The Effect of the Calcium Antagonist, Nimodipine, on Local Cerebral Blood Flow, Glucose Use and Focal Cerebral Ischaemia

By

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TO MY WIFE FAIRUZE

AND

MY DAUGHTER SUZANNE

ERRATA

1) Page 16 - line 1: Should read - ".... was inhibited by nifedipine."

2) Page 31 - line 5: Should read - ".... availability of calcium regardless of the agonist."

3) Page 51 - line 26: Should read - "The total area of the ipsilateral auditory cortex was 33664 picture points."

4) Page 55 - Table 12: The title should read - "The relationship between the CBF level and the cumulative area percent change obtained by densitometer."

5) Page 97 - new paragraph is added after line 6: "These tests assume that the blood flow data were normally distributed. Although this can never be certain from a small number of experiments, there is no indication that the distribution was skewed."

6) Page 153 - line 20: Should read - ".... whereas the region of the pons has markedly less binding sites of radiolabelled calcium antagonists."

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SUMMARY

Nimodipine (a dihydropyridine derivative) is one of a range of compounds which have been termed calcium antagonists, and whose pharmacological efficacy may result partly from an ability to inhibit the slow inward current of calcium ions in cell membranes. Nimodipine is a potent dilator of cerebral vessels in vitro and in situ, and this has stimulated interest in its effect on normal cerebral circulation and its use in cerebral ischaemia.

In the studies that form this thesis, I have examined several aspects of the effect of nimodipine on the cerebral circulatory responses in normal brain and in the presence of a well defined focal ischaemic lesion induced by occlusion of the middle cerebral artery (MCA).

The effect of the calcium antagonist, nimodipine, on local cerebral blood flow (CBF) in 31 regions of the CNS was studied with the [^{14}C]-iodoantipyrine autoradiographic technique in lightly anaesthetised, mechanically ventilated rats. Continuous intravenous infusion of nimodipine (1, 2 or 4 μg $kg^{-1}min^{-1}$) produced a dose-dependent reduction in mean arterial blood pressure (MABP) (reduced by 26 ± 2% after 30 min of nimodipine, 4 μg $kg^{-1}min^{-1}$) and a significant elevation in plasma glucose concentration (increased by 44 ± 2% after 30 min of nimodipine, 4 μg $kg^{-1}min^{-1}$). Local CBF was increased significantly during infusions of nimodipine (1 μg $kg^{-1}min^{-1}$) in 9 of the 31 regions examined, e.g., the parietal cortex (by 108%), sensory-motor cortex (by 132%), auditory cortex (by 78%), frontal cortex (by 65%). In contrast to the increases in CBF observed in forebrain regions, no significant

increases in CBF were observed during nimodipine infusion in regions of the lower brain stem, cerebellum and pons, or in myelinated fibre tracts. The proportionately greatest increases in local CBF were observed during infusion of the lowest dosage of nimodipine (1 μg $kg^{-1}min^{-1}$), suggesting either that this dosage provokes maximum cerebrovascular relaxation or that effects of increasing concentrations are counteracted by the concomitant systemic hypotension. The findings of this series of experiments emphasise that the dose must be regulated very carefully to avoid hypotension and hyperglycaemia; with calcium antagonists, correct dose selection may therefore be critical.

The effects of a continuous infusion of nimodipine (1 μg $kg^{-1}min^{-1}$) on local CBF and local cerebral glucose utilisation (CGU) were studied using the quantitative autoradiographic $[^{14}C]$-iodoantipyrine and $[^{14}C]$-2-deoxyglucose techniques in 34 anatomically discrete regions of the brain in lightly restrained, conscious rats. The infusion of nimodipine at this concentration produced only a small (8%) reduction in the MABP. The administration of nimodipine did not alter the rate of glucose utilisation in any of the regions examined. By contrast, in 24 regions CBF was increased significantly by 39-84% from control levels, most notably the cerebral cortices, thalamus, hypothalamus, most extrapyramidal regions, and some components of the limbic system, such as the hippocampus (molecular layer) and amygdala. Nimodipine did not alter CBF in the four white matter regions examined (mean increase in CBF: 1%). In vehicle treated animals there was an excellent correlation ($p<0.01$) between the local levels of CBF and glucose utilisation, with

the ratio of flow to glucose use being $\sim$1.5 ml μmol^{-1} in each brain region. During nimodipine treatment there was a similarly excellent correlation ($p < 0.001$) between local CBF and local CGU, but the median ratio between local flow and glucose use increased to 2.5 ml μmol^{-1}. Thus, the relationship between local CBF and local CGU was well maintained, but was reset towards a higher level of blood flow.

The effect of nimodipine (1 or 2 μg kg^{-1}min^{-1}) pre-treatment on local CBF ($[^{14}C]$-iodoantipyrine autoradiography) in rats that had undergone MCA occlusion were investigated in anaesthetised animals. In control animals, marked reductions in local CBF were observed throughout the cerebral cortices ipsilateral to MCA occlusion: the visual cortex (by 61%), auditory cortex (by 73%) and sensory-motor cortex (by 75%). The level of blood flow was reduced massively (by 92%) in the caudate nucleus ipsilaterally when compared to the contralateral side of the lesion. Nimodipine (1 μg kg^{-1}min^{-1}) pre-treatment increased the local CBF significantly in 26 of 34 regions of the contralateral hemisphere, particularly in the neocortical areas (range 76-96%; average 86%). The ipsilateral reductions in the local CBF observed in vehicle groups were significantly less pronounced with nimodipine (1 μg kg^{-1} min^{-1}) pre-treatment. The main areas in which the reduction in flow was minimised were the cortical regions (visual cortex, auditory cortex, parietal cortex, sensory-motor cortex and frontal cortex). By contrast, there were no changes in the local CBF in the centre of ischaemic lesion such as the caudate nucleus. To ensure that these measurements of CBF at predetermined neuroanatomically organised sites did not obscure

the analysis of the cerebrovascular actions of nimodipine, the autoradiograms were examined using a new approach to quantitative densitometry which involved analysis of the frequency distribution of CBF. This rigorous approach to densitometric analysis of the autoradiograms showed that nimodipine (1 $\mu g\ kg^{-1}\ min^{-1}$) pre-treatment reduced the amount of brain with low levels of CBF. For example, in animals receiving vehicle, the area of auditory cortex with CBF less than or equal to 25 ml $100g^{-1}min^{-1}$ was 10 $\pm$ 2% (contralateral) and 20 $\pm$ 3% (ipsilateral), and in nimodipine pre-treated groups the area of auditory cortex with CBF less than or equal to 25 ml $100g^{-1}min^{-1}$ was 8 $\pm$ 1% (contralateral) and 3 $\pm$ 1% (ipsilateral). Furthermore, analysis also showed that this pattern of flow distribution was not changed by nimodipine; instead, its effect was to maintain flow at a higher level throughout the hemisphere, and the higher values in the territory of the occluded artery formed simply a part of this general effect.

Nimodipine (2 $\mu g\ kg^{-1}min^{-1}$) pre-treatment increased the local CBF significantly in 10 cortical and subcortical areas of the brain when comparisons were made between the contralateral hemisphere with the data from control rats, e.g., auditory cortex (by 37%), parietal cortex (by 48%) and hippocampus (by 44%). When the hemisphere ipsilateral to occlusion was studied, nimodipine modified local CBF. The main areas in which the reduction in flow was minimised were the cortical regions, auditory and parietal cortices.

Neuropathological quantification of the ischaemic damage showed that nimodipine (1 $\mu g\ kg^{-1}\text{-}min^{-1}$) pre-treatment reduced the volume and the extent of cellular damage in the periphery,

but not the core of the lesion. The effects of the administration of nimodipine (1 $\mu g\ kg^{-1} min^{-1}$) (initiated 5 min after occlusion of the MCA) upon the local CBF have been studied in anaesthetised rats. Nimodipine post-treatment did not alter the level of local CBF in any of the 35 neuroanatomically defined areas either ipsilateral or contralateral to the occluded vessels. The lack of CBF alterations when nimodipine administration was initiated <u>after</u> occlusion of the MCA contrasted sharply with the elevations in CBF in the portions of the ischaemic hemisphere which occurred when nimodipine was administered <u>before</u> occlusion of the artery.

The results from this thesis represent a comprehensive description of the cerebrovascular effects of nimodipine in anaesthetised and conscious rats, and in a rat model of acute cerebral ischaemia.

PREFACE AND DECLARATION

This thesis deals with the effects of the calcium antagonist, nimodipine, upon various aspects of cerebrovascular responses in anaesthetised and conscious rats, and in rat models of focal ischaemic lesion.

The majority of the results of this thesis were obtained using the quantifiable autoradiographic [^{14}C]-iodoantipyrine technique. The Methods section describes in some detail the potential errors related to the measurement of the level of cerebral blood flow.

This thesis was obtained from my own original studies, and has not been presented previously as a thesis in any form.

CHAPTER I

INTRODUCTION

1. Historical Perspective

1.1 Calcium and contractile activity of smooth muscle.

More than a century ago, Sidney Ringer called attention to the importance of Ca^{++} in the contractions of muscle in his work on the contractile activity of isolated heart in the frog (1883). Later, Alexander Sandow (1952) suggested that Ca^{++} may play a key role in the muscle contraction. He proposed, as a working hypothesis, that "an action potential or membrane depolarization of the muscle-fibre surface promotes the entrance of Ca^{++} into the myoplasm, and this in turn provokes further reactions leading to the appearance of mechanical activity". Since that time, evidence has accumulated for this concept. In fact, Ca^{++} is the principal physiological ion that when injected at a low concentration into the muscle fibres produces shortening (Heilbrunn and Wiercinski, 1947; Niedergerke, 1955; Caldwell and Walster, 1963). A large bulk of information from several studies supports the view that Ca^{++} is the prime regulator of contraction-relaxation cycle in various types of muscles (Fatt and Katz, 1953; Hagiwara and Naka, 1964; Somlyo and Somlyo, 1968; Ebashi, 1980; Stull and Sandford, 1981; Weeb and Bohr, 1979; Tsien, 1983). In addition, the permeability of the cell membrane to calcium ion is of great importance for a variety of cellular functions such as secretion of neurotransmitters (Reuter, 1979; Duncan, 1983; Kraynack et al. 1983; Reuter, 1983).

1.2 Discovery of calcium antagonists.

In the late 1960s and early 1970s, a new group of extremely potent calcium antagonists were synthetised. In cardiac muscle during depolarisation, there is an initial fast current carried by Na^+ and a subsequent slow current carried partly by calcium ion (Coraboeuf, 1978; Reuter, 1979). Calcium penetrating into the cells through the slow channel appears to play an important role in triggering muscle contraction (Bolton, 1979). Albrecht Fleckenstein (1964) demonstrated that a newly synthesised coronary vasodilator mimicked the effects of simple withdrawal of calcium from the medium bathing isolated heart tissues. Fleckenstein had repeated Ringer's work (1883), and showed that the new compound, verapamil, inhibited cardiac contractibility, an effect which is reversible by calcium coupling excitation to contraction in heart muscle. Based upon such studies in cardiac, and later upon additional experiments in vascular smooth muscle (Fleckenstein et al. 1972; Fleckenstein, 1977; Godfraind, 1981), Fleckenstein and his colleagues, in 1969, introduced the term "calcium antagonists" to describe the compounds.

2. Terminology and Classification

2.1 Terminology.

Compounds which inhibit the influx of extracellular calcium into cardiac and vascular smooth muscle form a new and expanding class of therapeutic agents. Although originally referred to as "calcium antagonists" (Fleckenstein et al. 1969), other synonyms have been used; "slow-channel blockers", "calcium channel blockers", "calcium entry blockers", "calcium blockers",

"calcium influx antagonists", and so forth (Godfraind and Dieu, 1981; McCalden and Bevan, 1981; Vanhoutte, 1982; Janis et al. 1983; Norman et al. 1983; Sewing and Hannemann, 1983; Smith, 1983; Timmermans et al. 1983; Venter et al. 1983; Winquist and Baskin, 1983; Schwartz and Triggle, 1984). Furthermore, the term calcium antagonist has recently been used to describe compounds which inhibit contraction by effects upon intracellular calcium-dependent mechanisms; for example, trimethoxybenzoates or the methylenedioxyindene analogues (Rahwan et al. 1977, 1979; Plascik et al. 1978, 1979; Church and Zsoter, 1980; Rahwan, 1983; Weishaar et al. 1983). However, the extension in the use of this term has been criticised (Henry, 1980; Spedding, 1982; Kazda et al. 1983; Jones, 1984). It is highly likely, in view of their diverse chemical structure, that calcium antagonists possess different sites or cellular mechanisms of action. On the other hand, Fleckenstein (1982) argued that this is not a reason for abandoning the term calcium antagonist, which covers the therapeutic actions of these drugs more accurately than any of the alternative new names. In a strictly scientific sense these synonyms may be acceptable, but from a medical point of view they are misleading (Jones, 1984).

With the introduction of this new nomenclature, a re-assessment of the pharmacological properties of these drugs is required so that a clear distinction can be made between the calcium antagonists and compounds which modify the effects of calcium ions at intracellular or other additional sites. For the sake of clear terminology in this thesis, the name "calcium antagonist" is used to describe drugs which inhibit the influx of extracellular calcium into the cell.

2.2 Classification of calcium antagonists.

The potency of calcium antagonist drugs upon cardiac and smooth muscle has been used to sublassify these compounds. According to Fleckenstein (1982), calcium antangonists can be subdivided into two groups:

1) Group A: substances of outstanding calcium antagonistic potency and specificity, such as nimodipine, nifedipine, niludipine, verapamil, D600 and diltiazem.
2) Group B: Substances such as prenylamine, fendiline, terodiline, perhexiline, and caroverine. These drugs have prominent calcium antagonistic properties, but do not reach the outstanding degree of specificity of Group A.

The assessment of calcium antagonist drugs in high-K^+ depolarised intestinal smooth muscle (guinea pig taeniacoli) has also indicated that there are two main subtypes of drugs (Spedding, 1981, 1982).

A: The dihydropyridine class; for example, nimodipine, nifedipine, verapamil and diltiazem.

B: Flunarizine, cinnarizine, fendiline and pimozide.

These two main subdivisions agree with findings in vascular smooth muscle.

Recently, several studies have reported findings of the presence of ^3H dihydropyridine (nimodipine or nitrendipine)

binding sites in the rat (Ehlert et al. 1982; Murphy and Snyder, 1982; Murphy et al. 1983) and guinea pigs (Bellemann et al. 1981; Glossmann et al. 1982). The fact that there is excellent correlation between the ability of various dihydropyridines to displace specific ^{3}H-nitrendipine binding and to inhibit mechanical activity in the cell preparations (Weiss, 1981) is consistent with a specific locus of action for these compounds. Glossmann et al. (1982) have characterised these binding sites and have reached the conclusion that three classes of compounds exist.

> Class I: drugs which may possess a single binding site, such as fendiline, tiapamid and flunarizine, in addition to related dihydropyridine compounds (nimodipine and nifedipine).
>
> Class II: drugs which have been found to possess multiple sites; for example, verapamil and D600.
>
> Class III: drugs under the subdivisions of Class II drugs and diltiazem which increase ^{3}H-nimodipine binding (see also Ferry and Glossmann, 1982a,b).

It should be noted, however, that the grouping of flunarizine and fendiline with the dihydropyridine compounds is not consistent with the classification based pharmacological activity (Spedding, 1982) or the potency of the calcium antagonist drugs (Fleckenstein, 1982). This may be due to the possibility and flunarizine and fendiline are non-selective drugs and have additional cellular sites of action not detected by ligand and binding studies (Nakayama and Kusuya, 1980; Llenas and Massingham, 1983).

3. General Pharmacology

3.1 Pharmacology of calcium antagonists.

Calcium antagonists are a heterogeneous group of drugs which encompass structurally diverse compounds (Mizgala, 1983; Dipalmia, 1983; Singh, 1982, Nayler, 1983c). These drugs have different and variable effects on the contractibility of the myocardium and vascular smooth muscle (Fleckenstein, 1982).

An understanding of the pharmacokinetics of calcium antagonist drugs is essential in order to design appropriate dosage regimens which will provide optimum therapeutic efficacy. There are numerous studies regarding the kinetic of verapamil, diltiazem and nifedipine (Horster, 1975; Schlossmann et al. 1975; Rovei et al. 1977; Eichelbaum et al. 1979; Rovei et al. 1980; Kates, 1983; Meyer et al. 1983; Talbert and Bussey, 1983). Table 1 shows the major pharmacokinetic properties of these compounds. However, little is known about the pharmacokinetics of nimodipine.

3.2 Pharmacology of nimodipine.

3.2.1 General.

The calcium antagonist, nimodipine, was developed in the late 1970s and early 1980s (Meyer et al. 1983). Since then, several studies in animal and man have been performed to investigate its effect on vascular smooth muscle (Towart et al. 1981, 1982; White et al. 1982; Kazda et al. 1979, 1980, 1982), and cerebral blood flow in normal and ischaemic brain (Harper et al. 1981; Kazda et al. 1982; Gaab et al. 1982; Haws et al. 1983; Haws and Heistad, 1983, 1984; Steen et al. 1983; 1984; Smith

TABLE 1. PHARMACOKINETICS OF CALCIUM ANTAGONIST DRUGS.

Pharmacokinetics Feature	Calcium Antagonists		
	Verapamil	Nifedipine	Diltiazem
Oral absorption	90%	90%	90%
Half-life	3-7 hours	2-4 hours	4-9 hours
Metabolism	Liver	Liver	Liver
Plasma level	100-300 ng/ml	---	---
Protein binding	90%	90%	80%
Excretion	70% Renal 3-4% unchange	80% Renal 20% GIT	80% Renal 2-4% unchange
Average dosage	Oral: 40-120 mg 3-4 times daily	Oral: 10-60 mg 3 times daily	Oral: 60-120 mg 4 times daily
	I.V: 5-10 mg over 2 mins	I.V: 0.005- 0.15 mg/kg	I.V: 0.075- 0.15 mg/kg

From Dipalmia (1983).

et al. 1983). In these studies the dosages of nimodipine were variable with different schedules and route of administration. As mentioned previously (see Section 3.1), it is important to know the pharmacokinetics of nimodipine to assess its therapeutic efficacy. Thus, it is essential to use an appropriate dosage and schedule of administration. Accordingly, the plasma level of nimodipine was calculated to relate the results of the present studies to other in vitro investigations and to in vivo studies that employed different modes of administration.

3.2.2 Nimodipine chemistry.

Nimodipine is a dihydropyridine derivative (1,4-dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridine-dicarboxylic acid-isopropyl-(2-methoxy-ethyl)-ester) (Figure 1).

Nimodipine is a highly light-sensitive drug; thus, a special sodium vapour light is necessary during preparation of the drug.

3.2.3 Pharmacokinetic features of nimodipine.

Nimodipine is absorbed rapidly and completely after oral administration; the bio-availability of unchanged drug is 10% of the dose. The metabolites are excreted by renal and biliary routes. In rats, 80% of excretion is in the faeces and 20% in the urine. The half-life ($t_{1/2}$) of nimodipine is approximately 20 minutes for rats. Binding to plasma protein in rats is 96-98%. The distribution volume is approximately 0.7 L/kg for rats (personal communication, Dr. Kazda).

3.2.4 Concentration of nimodipine in the plasma.

When a drug is administered by continuous infusion

intravenously, the amount in the body will gradually increase to a plateau level (M) when the rate of elimination equals the rate of administration (Q).

that is:

$$M = \frac{Q \quad t\frac{1}{2}}{\text{In } 2} \qquad (1) \quad \text{Bowman and Rand, 1980)}$$

for nimodipine:

$$M^n = \frac{Q^n \; t^n\frac{1}{2}}{\text{In } 2}$$

where

M^n is the plateau level of nimodipine, $t^n\frac{1}{2}$ is the half-life of elimination for nimodipine, which is approximately 20 minutes for the rat.

Q^n the rate of administration; for example, 1 $\mu g \; kg^{-1} min^{-1}$.

Therefore,

$M^n = 28.9 \; \mu g \; kg^{-1}$.

Assuming 1 litre of blood equals approximately 1.05 kg (Sakurada et al. 1978), $M^n = 27.5 \; \mu g \; L^{-1}$.

The molecular weight of nimodipine is 418.5.

$$M^n = \frac{27.5 \times 10^{-6}}{418.5} = 66 \text{ nM.}$$

The time reaches a given fraction (F) of plateau level is given by

$F = 1 - e^{-} (t/t\frac{1}{2}) \text{ in } 2 \qquad (2)$ Bowman and Rand, 1980).

$$t^n = \frac{\text{Log}_e (1-F) \; t\frac{1}{2}}{\text{Log}_e 2} \qquad (3)$$

where t^n = time to reach the plateau for nimodipine.

Assuming F = 0.99

$$t^n = \frac{4.605 \times 20}{0.693} \qquad (4)$$

$$= 133 \text{ minutes.}$$

For equation (4) a different level of (F) according to time per minute can be calculated (see Table 2, Figure 2). In the same

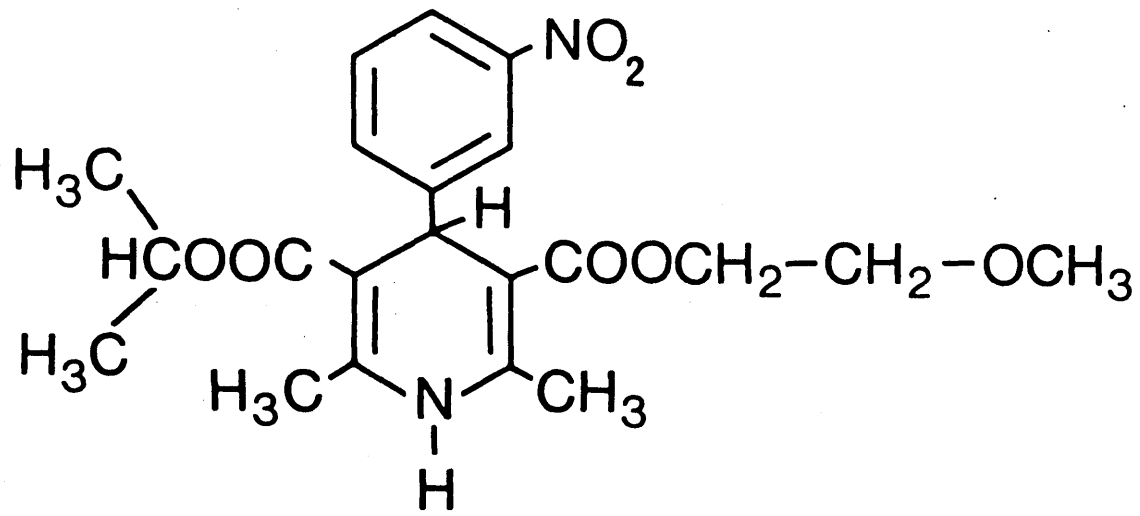
time the concentration of nimodipine in the plasma at different time per minute can be determined by equation (1,4) (see Figure 2).

3.2.5 Concentration of nimodipine in the CNS.

It has been estimated that the mean concentration level of nimodipine was 6.9 $\pm$ 4.9 ng per millilitre in plasma and 0.77 $\pm$ 0.34 ng per millilitre in cerebrospinal fluid (Allen et al. 1983). In the rat, 96-98% of nimodipine is bound to protein in the plasma; the plasma concentration of nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) at 60 minutes when infused intravenously was 58 nM (see Figure 2). The free concentration of nimodipine will be 1.7 nM, and the approximate CNS level is 1.74 nM. Andersson et al. (1983) suggested that the IC_{50} (the concentration of drug required to produce 50% of relaxation) for nimodipine is 1.9 $\pm$ 1.5 nM in the cat, which is approximately the same as the rat (Edvinsson, personal communication) which is very close to the level of nimodipine reached in the CNS (1.7 nM).

Figure 1.

THE CHEMICAL STRUCTURE OF NIMODIPINE



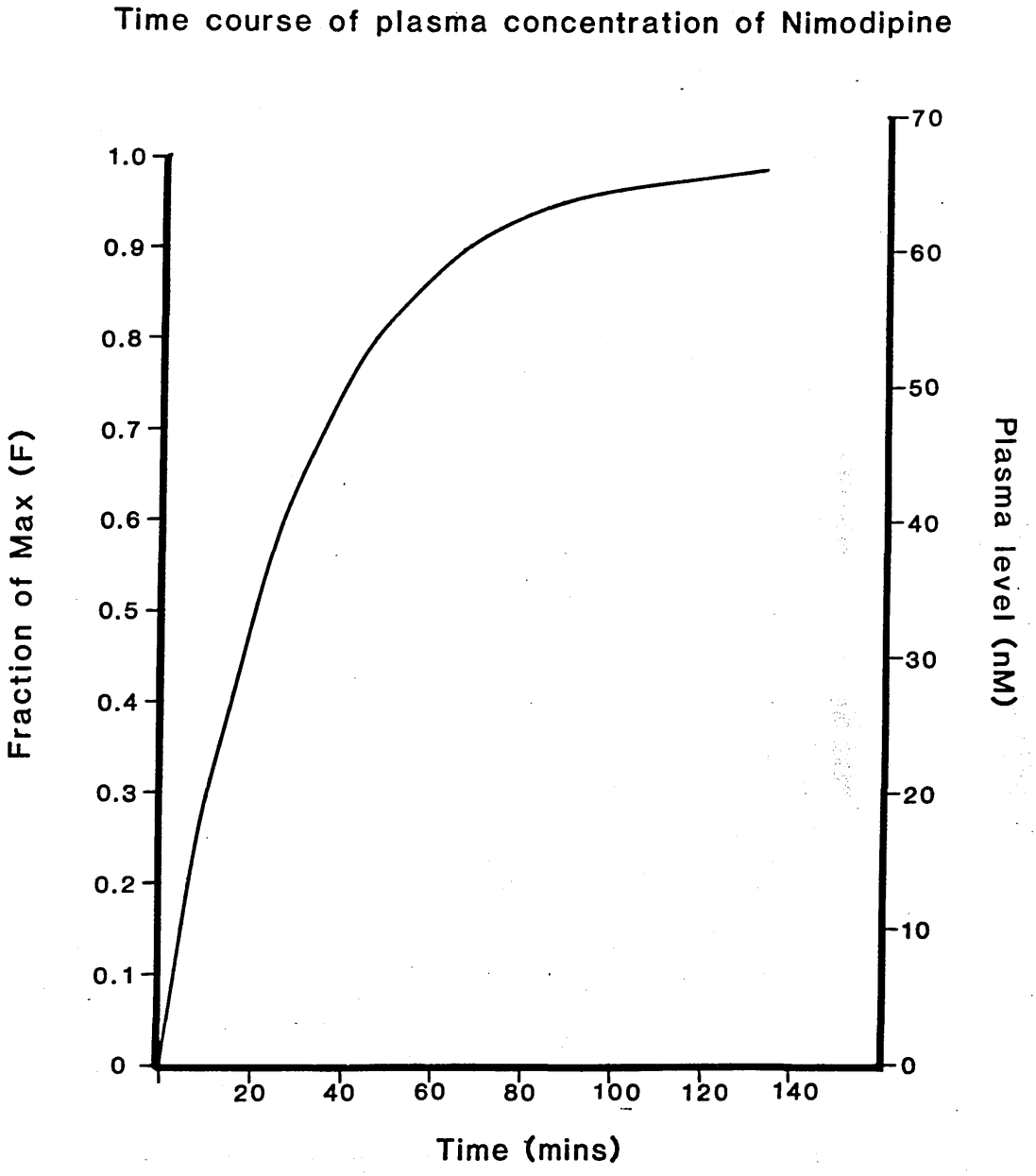
Nimodipine = Bay e 9736

TABLE 2. TIME COURSE OF NIMODIPINE IN PLASMA (1 $\mu g\ kg^{-1} min^{-1}$, IV).

CSF nM	Time (mins)	Fraction of Max F	(1 - F)	Log_e	Plasma Level nM
1.98	133	0.99	0.01	4.605	66
1.88	86.5	0.95	0.05	2.999	62.7
1.78	66	0.90	0.1	2.302	59.4
1.58	46	0.80	0.2	1.609	52.8
1.38	35	0.70	0.3	1.204	46.2
1.19	26	0.60	0.4	0.916	39.6
0.99	20	0.50	0.5	0.693	33
0.79	15	0.40	0.6	0.519	26.4
0.59	10	0.30	0.7	0.356	19.8
0.40	6	0.20	0.8	0.223	13.2
0.20	3	0.10	0.9	0.105	6.6

Different level of fraction of max (F) according to time per min is calculated from equation 4, and the plasma level of nimodipine at different time per min is calculated from equations 4 and 1 (see Section 3.2.4).

Figure 2.



Time course of plasma concentration of nimodipine (1 $\mu g\ kg^{-1}\ min^{-1}$, i.v.). The time to reach the plateau for nimodipine is 133 minutes.

4. Calcium Antagonists and Vascular Smooth Muscle

4.1 Calcium channels.

Vascular smooth muscle is a heterogeneous tissue and calcium movements during contractile process are complex (Bohr, 1973; van Neuten and Vanhoutte, 1981; Gillis, 1982). The concentration of calcium which raises intracellular free Ca^{++} to initiate contraction depends on the nature of pharmacological stimulus (Winquist et al. 1981) and the phase of the actual contraction studies (Bohr, 1963; Sitrin and Bohr, 1971; Godfraind and Kaba, 1972; Godfraind, 1981). Bolton (1979) proposed an explanation for the calcium movement in vascular smooth muscle cells. He suggested that there are at least two different calcium channels in the cell membrane which permits the calcium to enter the cell.

1) The potential operated channel (POC), which is an ion channel population that opens as the potential across the membrane is reduced; for example, after K^{+}-depolarisation.

2) The receptor operated calcium channel (ROC), which is an ion channel controlled or operated by the receptor for a particular stimulant substance; for example, noradrenaline.

4.2 Variability of sensitivity of POCs and ROCs to calcium antagonists.

The relaxant effect of calcium antagonists has been demonstrated in many types of vascular smooth muscle (see Tables 3-7). The selectivity of calcium antagonists in different parts of the circulatory system appears to be related to differences in the

calcium sources and type of calcium channels used to mediate Ca^{++} influx (Toda, 1974; Golenhofen and Hermstein, 1975; Bolton, 1979; McCalden and Bevan, 1981).

Bohr (1963) divided responses of rabbit aorta to noradrenaline into two components, an initial fast phasic response (Phase I) and a later slow or tonic response (Phase II). The time course of the tonic response depended directly on the extracellular concentrations of Ca^{++} and was sensitive to calcium antagonists (Bevan et al. 1982). The concept that the POC is more sensitive to calcium antagonists than the ROC is supported by the findings that in most vascular tissues K-induced contractions are more sensitive to calcium antagonists than noradrenaline (NA)-induced contractions (see Tables 3-7).

In the rabbit aorta, K-induced contractions have been shown to be more sensitive to inhibition by the calcium antagonist, nisoldipine (Kazda et al. 1980), D600 (Schumann et al. 1975; Kazda et al. 1980), diltiazem (van Breemen et al. 1981), cinnarizine (Brockaert and Godfraind, 1979) and nifedipine (Schumann et al. 1975) than NA-induced contractions. Similar results have been obtained for D600 in rabbit mesenteric (Schumann et al. 1975), basilar and ear arteries (McCalden and Bevan, 1981); for diltiazem in rabbit renal artery (Bevan, 1982); for diltiazem and nifedipine in rat portal vein (Church and Zsoster, 1980) and in the perfused rat mesenteric vascular bed (Kondo et al. 1980); for cinnarizine (Brockaert and Godrained, 1979) and nisoldipine (Saida and van Breemen, 1983) in rabbit mesenteric artery; for nifedipine and flunarizine in rat mesenteric artery (Godfraind and Miller, 1982). Furthermore, contractions induced by prostaglandin ($PGF_{2\alpha}$) in the human pial artery, and dog coronary

to inhibition by nifedipine (Brandt et al. 1981a), and verapamil (Shimizu et al. 1980), respectively, that is activation by depolarisation.

However, it may be argued that the ROC sensitivity to calcium antagonists cannot be directly deduced from these results, since Na-induced Ca^{++} influx through the ROC may cause secondary membrane depolarisation and consequently POC activation (Cauvin et al. 1983).

In contrast, numerous investigations indicate that the ROC is more sensitive to calcium antagonists than the POC in certain vascular tissues (see Tables 3-7). Walus et al. (1981) have shown that K^+-contracted strips of isolated canine mesenteric arteries are less sensitive to nifedipine than are NA-contracted strips. Rat superior mesenteric arterial strips contracted with a K^+-depolarising solution are shown to have similar sensitivity to cinnarizine as compared with NA-contracted strips. Bevan (1982) has demonstrated that in the rabbit basilar artery NA-induced contractions are more sensitive to diltiazem than K^+-induced contractions, whereas they are about the same in the ear and mesenteric arteries. Verapamil is about equi-sensitive in inhibiting K^+- and NA-induced contractions of the perfused mesenteric vascular bed of the rat (Kondo et al. 1980), and in inhibiting $PGA_{2\alpha}$ and K^+-induced contraction of the dog cerebral artery (Shimizu et al. 1980).

Furthermore, Towart et al. (1981) suggested that the sensitivity of basilar artery in rabbit to the calcium antagonist, nimodipine, is equi-sensitive to 5-HT (5-hydroxytryptamine) and K^+-induced contraction, since the IC_{50} for both vasoconstrictive substances was 2×10^{-10}M. They postulated

that the selectivity of nimodipine was due to blockade of the ROC in these vessels. Cauvin et al. (1982) have demonstrated that diltiazem inhibition to NA and high K^+-induced contractions showed IC_{50} values of 1×10^{-8} and 6×10^{-7}M, respectively, indicating that the ROC was more sensitive than POC to actions of diltiazem.

In conclusion, calcium antagonists do not readily block ROCs that admit calcium, but do block the POCs (Bolton, 1979; Nayler and Pool-Wilson, 1981; Godfraind, 1981, 1982, 1983; Högestatt et al. 1982; Bou et al. 1983; Nayler and Horowitz, 1983; Andersson and Högestatt, 1984). However, other studies suggested that calcium antagonists also may interfere with ROCs in certain vascular smooth tissues (Towart, 1981; Cauvin et al. 1982; Bevan, 1982; Godfraind and Miller, 1982).

This variability in the concept of selectivity of calcium antagonists either on POCs or ROCs may be due to the following observations:-

1) There are regional as well as species variations in sensitivity to calcium antagonists.
2) The variability of the calcium pool among vascular smooth muscles.
3) The potency of these drugs for inhibiting K or agonist-induced contractions varies widely in vascular smooth muscles.

4.3 Selectivity of calcium antagonists on cerebral vascular smooth muscle.

Cerebrovascular smooth muscle differs from other peripheral vascular smooth muscle. Moreover, there is a marked

species variation in cerebral vessels (Duckles et al. 1977; Edvinsson and MacKenzie, 1977; Heistad et al. 1978). It follows that the consequences of calcium antagonists on vascular smooth muscle, either in vitro, and particularly in vivo, cannot be extrapolated from studies of other regional beds, even in the same species and certainly not between species (Bevan et al. 1982). This variability, however, may not exclude the possibility of considerable pharmacologic selectivity of calcium antagonist action on cerebral vessels. Several comparative studies have shown that calcium antagonists attenuate the contraction of cerebral vessels provoked by a wide range of pharmacological (e.g., noradrenaline, serotonin, prostaglandin) and pathological stimuli (e.g., blood, cerebral ischaemia) (Hayashi and Toda, 1977; Allen and Banghart, 1979; Brandt et al. 1979, 1981a,b, 1983; Tanaka et al. 1980; Auer et al. 1981; Towart and Kazda, 1980; Auer et al. 1982, 1983; Högestatt et al. 1982; White et al. 1982; Andersson et al. 1983).

Such selectivity has been described by Allen and Banghart (1979), who demonstrated the preferential action of nifedipine on cerebral vessels compared with femoral arteries of dog (see Table 4). Relaxations provoked by diltiazem in basilar artery were more sensitive to calcium antagonists than either mesenteric or the ear arteries of rabbit. Shimizu et al. (1980) have shown that the response of the dog basilar artery to prostaglandin $F_{2\alpha}$ was more sensitive to the vasodilatatory action of diltiazem, and of verapamil and nifedipine (see Tables 4 and 5). The topical application of nifedipine induces relaxation of feline cerebral arterioles that have been previously constricted by perivascular

injections of blood (Brandt et al. 1979). Nifedipine is more potent in relaxing the basilar than the facial artery (Högestatt et al. 1982), and cerebral vessels were shown to be more sensitive to NA- and K^+-induced contraction than the ear artery in rabbit (McCalden and Bevan, 1981, 1982). Nimodipine is a potent calcium antagonist, and it has been shown to have greater effects on cerebral than peripheral arteries (Towart and Kazda, 1979, 1980, 1982; Brandt et al. 1981a,b; Kazda and Towart, 1981; Towart and Perzborn, 1981; Towart, 1982; Andersson et al. 1983).

Nimodipine and other calcium antagonists effectively inhibit the serotonin induced contractions of isolated rings of the rabbit basilar and saphenous arteries. The order of potency of the calcium antagonist drugs was nimodipine, nifedipine, verapamil (Towart et al. 1982; Towart and Kazda, 1982). Andersson et al. (1983) demonstrated that the order of potency for the relaxation of human cerebral and mesenteric arteries of the various calcium antagonist drugs was nimodipine, nifedipine, verapamil and diltiazem.

White et al. (1982) demonstrated that nimodipine effectively vasodilated the contractile responses induced in isolated canine basilar arteries by serotonin, prostaglandin $F_{2\alpha}$, thrombine and whole blood. Nimodipine was more potent than other dihydropyridine or non-dihydropyridine calcium antagonists in relaxing the contractile activation induced by various stimuli. By contrast, Brandt et al. (1981a) showed that nifedipine was more potent than nimodipine in relaxing human cerebral arteries contracted by potassium.

Cerebrovascular smooth muscle appears to be more sensitive than other vessels to the action of calcium antagonists.

This may be due to the fact that the intracellular calcium pool is smaller in cerebral vessels than other vascular beds (Edvinsson et al. 1979; Bevan, 1981; McCalden and Bevan, 1981; Towart et al. 1981; Cauvin et al. 1983; Bou et al. 1983).

TABLE 3.

EFFECT OF THE CALCIUM ANTAGONIST, NIMODIPINE,

ON VASCULAR SMOOTH MUSCLE.

Species	Vessels compared	Contractile activation, agent and the IC_{50}		Reference
		NA	K^+	
Rabbit	Aorta	3×10^{-5}	6×10^{-9}	Towart et al. (1982)
		5HT		
Rabbit	Basilar artery	2×10^{-10}	2×10^{-10}	Towart et al. (1982)
	Saphenous artery	2×10^{-6}	3×10^{-10}	
		TXA_2		
Rabbit	Basilar artery	2×10^{-9}	-	Towart and Perzborn (1981)
	Saphenous artery	7×10^{-8}	-	
		$PGF_{2\alpha}$		
Human	Pial artery	2×10^{-8}	2×10^{-9}	Brandt et al. (1981a)
	Mesenteric artery	-	7×10^{-9}	
		$PGF_{2\alpha}$		
Cat	Middle cerebral artery	3×10^{-9}	2×10^{-9}	Andersson et al (1983)
	Mesenteric artery	-	1×10^{-8}	

IC_{50} (concentration of the drug to produce 50% relaxation or inhibition of contraction, M); K^+ (potassium); NA (noradrenaline); 5HT (5-hydroxytryptamine); TXA_2 (Thromboxane A_2); $PGF_{2\alpha}$(prostaglandin).

TABLE 4.

EFFECT OF THE CALCIUM ANTAGONIST, NIFEDIPINE,

ON VASCULAR SMOOTH MUSCLE.

Species	Vessels compared	Contractile activation, agent and the IC_{50}		Reference
		NA	K^+	
Human	Mesenteric artery	2×10^{-6}	1×10^{-7}	Mikkelsen et al. (1978)
	Mesenteric vein	3×10^{-6}	1×10^{-7}	
		5HT		
Dog	Basilar artery	8×10^{-8}	4×10^{-2}	Allen and Banghart (1979)
	Femoral artery	5×10^{-7}	5×10^{-2}	
		NA		
Dog	Basilar artery	2×10^{-6}	-	Allen and Banghart (1979)
	Femoral artery	3×10^{-6}	-	
		$PGF_{2\alpha}$		
Dog	Cerebral artery	3×10^{-8}	-	Shimizu et al. (1980)
	Mesenteric artery	5×10^{-7}	-	
		$PGF_{2\alpha}$		
Human	Pial artery	2×10^{-8}	7×10^{-9}	Brandt et al. (1981a)
	Mesenteric artery	-	2×10^{-8}	
		$PGF_{2\alpha}$		
Cat	Middle cerebral artery	3×10^{-8}	7×10^{-9}	Andersson et al. (1983)
	Mesenteric artery	-	2×10^{-8}	
		NA		
Rat	Aorta	2×10^{-8}	1×10^{-9}	Godfraind (1982)
	Mesenteric artery	4×10^{-8}	2×10^{-9}	

IC_{50} (concentration of the drug to produce 50% relaxation or inhibition of contraction, M); K^+ (potassium); NA (noradrenaline); 5HT (5-hydroxytryptamine); $PGF_{2\alpha}$(prostaglandin).

TABLE 5.

EFFECT OF THE CALCIUM ANTAGONIST, VERAPAMIL,

ON VASCULAR SMOOTH MUSCLE.

Species	Vessels compared	Contractile activation, agent and the IC_{50}		Reference
		NA	K^+	
Human	Mesenteric artery	3×10^{-6}	3×10^{-6}	Mikkelsen et al. (1978)
	Mesenteric vein	3×10^{-6}	4×10^{-6}	
		NA		
Rat	Mesenteric bed	6×10^{-6}	6×10^{-6}	Kondo et al. (1980)
	Aorta	4×10^{-6}	3×10^{-7}	Massingham (1973)
		$PGF_{2\alpha}$		
Dog	Cerebral artery	1×10^{-7}	1×10^{-7}	
	Coronary artery	6×10^{-7}	3×10^{-7}	Shimizu et al. (1980)
	Mesenteric artery	2×10^{-7}	2×10^{-7}	
		$PGF_{2\alpha}$		
Cat	Middle cerebral artery	4×10^{-7}	2×10^{-7}	Andersson et al. (1983)
	Mesenteric artery	-	5×10^{-7}	

IC_{50} (concentration of the drug to produce 50% relaxation or inhibition of contraction, M); K^+ (potassium); NA (noradrenaline); $PGF_{2\alpha}$ (prostaglandin).

TABLE 6.

EFFECT OF THE CALCIUM ANTAGONIST, DILTIAZEM, ON VASCULAR SMOOTH MUSCLE.

Species	Vessels compared	Contractile activation, agent and the IC_{50}		Reference
		NA	K^+	
Rabbit	Basilar artery	1×10^{-8}	1×10^{-7}	Bevan (1982)
	Ear artery	1×10^{-6}	2×10^{-6}	
		NA		
Rabbit	Mesenteric artery	2×10^{-7}	6×10^{-7}	Bevan (1982)
	Saphenous vein	2×10^{-7}	-	
		5HT		
Rabbit	Basilar artery	8×10^{-8}	1×10^{-7}	Bevan (1982)
	Ear artery	8×10^{-7}	2×10^{-6}	
		NA		
Rabbit	Mesenteric artery	1×10^{-8}	6×10^{-7}	Cauvin et al. (1982)
		$PGF_{2\alpha}$		
Cat	Middle cerebral artery	4×10^{-7}	1×10^{-6}	Andersson et al. (1983)
	Mesenteric artery	-	3×10^{-6}	
		NA		
Rabbit	Mesenteric artery	5×10^{-7}	6×10^{-7}	Cauvin et al. (1984)
	Aorta	3×10^{-4}	3×10^{-7}	

IC_{50} (concentration of the drug to produce 50% relaxation or inhibition of contraction, M); K^+ (potassium); NA (noradrenaline); 5HT (5-hydroxytryptamine); $PGF_{2\alpha}$ (prostaglandin).

TABLE 7.

EFFECT OF THE CALCIUM ANTAGONIST, D600,

ON VASCULAR SMOOTH MUSCLE.

Species	Vessels compared	Contractile activation, agent and the IC_{50}		Reference
		NA	K^+	
Rat	Aorta	7×10^{-8}	2×10^{-8}	Massingham (1973)
Rabbit	Mesenteric artery	4×10^{-5}	5×10^{-8}	Schumann et al. (1975)
Dog	Coronary artery	1×10^{-6}	1×10^{-7}	Van Breemen and Siegel (1980)
Rabbit	Aorta	6×10^{-6}	1×10^{-7}	Meisheri et al. (1981)
Rabbit	Basilar artery	6×10^{-8}	5×10^{-9}	McCalden and Bevan (1981)
	Ear artery	6×10^{-7}	4×10^{-9}	
		5HT		
Rabbit	Basilar artery	3×10^{-8}	-	McCalden and Bevan (1981)
	Ear artery	3×10^{-7}	-	

IC_{50} (concentration of the drug to produce 50% relaxation or inhibition of contraction, M): K^+ (potassium); NA (noradrenaline); 5HT (5-hydroxytryptamine).

5. Ligand Binding Studies of Calcium Antagonists

Calcium antagonists can be labelled with [^{3}H]-nitrendipine (Bellemann et al. 1981, 1982; Bolger et al. 1982, 1983; Ehlert et al. 1982; Gould et al. 1982; Murphy and Snyder, 1982), [^{3}H]-nimodipine (Bellemann et al. 1982; Ferry and Glossmann, 1982a; Glossmann et al. 1982, 1983a; Ferry et al. 1984), [^{3}H]-nifedipine (Holck et al. 1983; Pan et al. 1983), [^{3}[^{3}H] Bay kpine (Pan et al. 1983) and the calcium agonist [^{3}H] Bay k 9644 (Janis et al. 1984a,b).

High affinity binding sites for the dihydropyridine calcium antagonists have been recently described in various tissue membranes (see Tables 8 and 9), including brain, heart, vascular smooth muscle and skeletal muscle. The dihydropyridine derivatives interacted competitively and with high potency at the same receptor site (Ehlert et al. 1982; Janis and Triggle, 1984), whereas verapamil and D600 were somewhat less potent and interacted at a site distinct from the dihydropyridine binding site (Ehlert et al. 1982; Glossmann and Ferry, 1983a,b; Janis and Triggle, 1984). The non-dihydropyridine class of calcium antagonists do not compete directly at these sites, but do increase the dissociation rate of [^{3}H]-nitrendipine in cerebral cortex (Murphy et al. 1983); verapamil and prenylamine decrease [^{3}H]-binding, whereas diltiazem increases [^{3}H]-binding (Ehlert et al. 1982; Depover et al. 1983; Glossmann et al. 1983a,b; Murphy et al. 1983).

Nimodipine, nitrendipine, nisoldipine and nifedipine are more potent in inhibiting the [^{3}H]-nitrendipine binding sites in brain and heart membranes than verapamil, D600 and diltiazem (Ehlert et al. 1982; Ferry et al. 1983a,b; Holck et al. 1983;

Peroutka and Allen, 1983). The relative potencies of various dihydropyridine agents in competing for binding sites (Bellemann et al. 1981; Bolger et al. 1982) parallel their pharmacological potencies (Henry, 1980; Fleckenstein, 1982).

Collectively, the radio ligand studies indicate the existence of high-affinity binding sites both for $[^3H]$-nimodipine and other agents of 1,4 dihydropyridines and for non-dihydropyridine calcium antagonists, in cardiac and vascular smooth muscle, skeletal muscle and brain. Furthermore, the radiolabelled ligand studies have been used to identify the calcium channels in various types of tissue. However, it should be noted that many of these studies were performed with homogenates from fully innervated organs, thus leaving the possibility open that nerve endings contribute to the overall binding. In experiments using rat brain it was observed that $[^3H]$-nitrendipine binding sites were localised to areas of the brain rich in synaptic connections and were largely confined to neural elements (Murphy and Snyder, 1982).

TABLE 8.

[^{3}H]-NIMODIPINE BINDING SITES TO TISSUE MEMBRANES.

Species	Tissue	Kd	B Max	Reference
Porcine	Coronary artery	1.5	0.10	Dompert and Traber (1984)
Porcine	Pial vessels	1.9	0.20	Dompert and Traber (1984)
Porcine	Ventricle	1.4	0.18	Dompert and Traber (1984)
Rat	Ventricle	0.25	0.39	Janis et al. (1982)
Guinea-pig	Ventricle	0.35	0.4	Glossmann and Ferry (1983a)
Guinea-pig	Skeletal muscle	1.5	2.0	Ferry and Glossmann (1982a)
Guinea-pig	Brain	0.3-0.4	0.3-0.35	Bellemann et al. (1982)
Rat	Cerebral cortex	1.1	0.50	Bellemann et al. (1983)
Guinea-pig	Brain	0.4-0.8	0.60	Glossmann et al. (1983b)
Guinea-pig	Brain	0.52	0.58	Glossmann and Ferry (1983a)
Human	Brain	0.27	0.39	Peroutka and Allen (1983)

Kd (nM) represents the dissociation constant, and B max (pmol.mg^{-1} of protein) is the maximal number of sites.

TABLE 9.

$[^3H]$-NITRENDIPINE BINDING SITES TO TISSUE MEMBRANES.

Species	Tissue	Kd	B Max	Reference
Bovine	Aorta	0.16	0.080	Sarmiento et al. (1984)
Canine	Aorta	0.3	0.020	Triggle et al. (1982)
Rabbit	Aorta	1.0	0.054	Bristow et al. (1982)
Porcine	Coronary artery	1.0	0.035	De Pover et al. (1982)
Canine	Mesenteric artery	0.25	0.025	Triggle et al. (1982)
Rat	Mesenteric artery	0.10	0.018	Triggle et al. (1982
Canine	Atria	0.14	0.170	Sarmiento et al. (1982)
Canine	Ventricle	0.30	1.5	Williams and Jones (1983)
Guinea-pig	Ventricle	0.1	0.3	Bellemann et al. (1981)
Rabbit	Ventricle	0.19	0.25	Janis et al. (1984b)
Guinea-pig	Ventricle	0.16	0.213	Gould et al. (1983)
Rabbit	Skeletal muscle	0.75	3.18	Janis et al. (1984a)
Guinea-pig	Skeletal muscle	3.6	7.0	Ferry et al. (1983a)
Guinea-pig	Skeletal muscle	2.28	1.11	Gould et al. (1983)
Rabbit	Skeletal muscle	1.0	6.7	Fairhurst et al. (1983)

Kd (nM) represents the dissociation constant, and B max (pmol.mg^{-1} of protein) is the maximal number of sites.

6. Calcium Antagonists and Cardiovascular Circulation

In the last twenty years much work has been devoted to emphasising the effect of calcium antagonists on cardiovascular circulation. For example, verapamil (Sandler et al. 1968; Livesley et al. 1973; Andreasen et al. 1975; Rosing et al. 1979a; Ferilinz, 1981) and nifedipine (Kobayashi et al. 1972; Ebner, 1975; Lorell et al. 1980) have been shown to be of clinical value in the treatment of angina pectoris. Both drugs increase exercise tolerance in patients suffering from angina pectoris (Previtali et al. 1980; Johnson et al. 1981). Verapamil (Rosing et al. 1979b) and nifedipine (Klugmann et al. 1980) reduce blood pressure and total peripheral resistance, and it has been found that both drugs may increase coronary flow in the isolated perfused heart (Hashimoto et al. 1972; Refsum, 1975). Recently, calcium antagonists represent a major therapeutic breakthrough in the treatment of various cardiovascular disorders; for example, angina (Theroux et al. 1983), atrial flutter and fibrillation (Brisse et al. 1982), hypertension (Buhler et al. 1983; Spivack et al. 1983), supraventricular tachycardia and ventricular tachyarhythmia (Singh et al. 1983). Furthermore, intensive studies on the effects of calcium antagonists on cardiovascular circulation proved that these drugs possess a great potential in the treatment of aortic insufficiency (Fioretti et al. 1982), atherosclerosis (Henry, 1980, 1982), cardioplegia (Clark et al. 1982), hypertensive emergency (Bertel et al. 1983), hypertrophic cardiomyopathy (Landmark et al. 1982; Spicer et al. 1983), migraine (Diamond and Schenbaum, 1983; Kahan et al. 1983), and myocardial ischaemia and failure (Bellocci et al. 1982; Winniford et al. 1982).

7. The Effects of Calcium Antagonists on Cerebral Circulation

The effects of calcium antagonists on the cerebral circulation in intact animals have not been clearly established. Various authors suggested that calcium antagonists prevent cerebral arterial constriction by affecting the intracellular availability of calcium, regardless the agonist. There is almost universal agreement that nimodipine and other calcium antagonists attenuate the contraction of cerebral vessels provoked by a wide range of pharmacological (e.g., noradrenaline, serotonin, protaglandin) and pathological stimuli (e.g., blood, cerebral ischaemia) in vitro (Allen and Banghart, 1979; Towart and Kazda, 1979, 1982; Shimizu et al. 1980; Kazda and Towart, 1981; Towart and Perzborn, 1981; White et al. 1982; Andersson et al. 1983; van Neuten et al. 1983) and in vivo (Allen and Bahr, 1979; Cohen and Allen, 1980; Tanaka et al. 1980; Auer, 1981; Brandt et al. 1981b; Auer et al. 1982; Boisvert, 1983).

Several studies described marked elevations in CBF following the administration of calcium antagonists (Harper et al. 1981; Kazda et al. 1982; Haws et al. 1983). Furthermore, some investigators showed that calcium antagonists have a protective effect in several animal models of cerebral ischaemia (Hoffmeister et al. 1979; Kazda et al. 1979; Steen et al. 1983). Allen and Bahr (1979) injected 2.5 ml of whole blood into the subarachnoid space in dogs and showed that the nifedipine (1 mg kg^{-1}) pre-treatment effectively prevented the acute spasm. The results of this study were confirmed in patients by randomised, double-blind, placebo

controlled trial of nimodipine (Allen et al. 1983). One hundred and twenty five patients with intracranial aneurysm were treated within 96 hours of their subarachnoid haemorrhage, which was documented by CAT scanning or lumbar puncture. Overall, nimodipine significantly reduced the occurrence of severe neurologic deficits due to spasm. It was felt that the clinical efficacy of nimodipine was the result of its inhibition of cerebral arterial spasm.

By contrast, several studies doubted the beneficial effect of calcium antagonists in preventing cerebral infarction (Harris et al. 1982), and others were unable to demonstrate any significant change in the CBF (Edvinsson et al. 1983; Newberg et al. 1984).

The level of cerebral oxidative metabolism is a crucial determinant of the level of CBF under normal circumstances (Kuschinsky and Wahl, 1978; McCulloch, 1982), and the effects of many pharmacological agents on cerebral metabolism may be the cause of changes in CBF rather than a direct action of the agent on cerebral vessels (Edvinsson and MacKenzie, 1977; Kuschinsky and Wahl, 1978). D'Avella et al. (1984) have studied the effect of the calcium antagonist on the cerebral metabolism and found that nimodipine increased glucose use in all brain regions. However, global cerebral oxygen consumption did not appear to be altered during nimodipine administration in the studies of Harper et al. (1981); Haws et al. (1983); Steen et al. (1983); McCalden et al. (1984). On the other hand, the recent demonstrations of highly specific dihydropyridine binding sites in the brain (Bellemann et al. 1982, 1983; Ferry and Glossmann, 1982a,b; Quirion, 1983) and

the behavioural alterations after the administration of calcium antagonists (Hoffmeister et al. 1982; Shah et al. 1983), indicate that nimodipine could act on cerebral tissue itself in addition to any actions it may exert on cerebral vessels.

8. Vasodilators and Calcium Antagonists in the Treatment of Cerebral Ischaemia

Stroke is defined (World Health Organisation (WHO) as recommended by Marquardsen, 1978) as a rapidly developed sign of focal (or global) disturbance of cerebral function, leading to death, or lasting more than 24 hours, with no apparent cause other than vascular origin. A similar definition for stroke was adopted by the Royal College of Physicians (RCP) working group on stroke (1974).

The yearly incidency of stroke is approximately 2 per 2,000 per year for all ages (Kurtzke, 1976). Harris et al. (1971) found that stroke is the commonest cause of severe physical disability, and estimated that up to 130,000 people living at home in the United Kingdom are handicapped to some degree by stroke.

Various therapeutic regimens have been suggested to protect the brain during an ischaemic insult. Two main approaches have been explored in attempts to alleviate the consequences of cerebral ischaemia; in one, the primary target is the neuron itself; in the other, the aim is primarily to influence cerebral blood flow (CBF). Thus, the outcome of an ischaemic insult may be improved by increasing the tolerance of neuronal cells to ischaemic insult, or by the prevention of CBF falling below a critical threshold. Each mechanism may be influenced

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Various therapeutic regimens have been suggested to protect the brain during an ischaemic insult. Two main approaches have been explored in attempts to alleviate the consequences of cerebral ischaemia; in one, the primary target is the neuron itself; in the other, the aim is primarily to influence cerebral blood flow (CBF). Thus, the outcome of an ischaemic insult may be improved by increasing the tolerance of neuronal cells to ischaemic insult, or by the prevention of CBF falling below a critical threshold. Each mechanism may be influenced

by calcium antagonists. Calcium is involved in the evolution of irreversible damage at the cellular level, and in vasomotor events in cerebral vessels (Siesjö, 1981, 1984).

It has been observed that barbiturates decreased the metabolic rate of oxygen consumption ($CMRO_2$), and reduced significantly the size of infarcts in dogs following middle cerebral artery occlusion (Smith et al. 1974; Molinari et al. 1976; Smith and Marque, 1976; Simeone et al. 1979). However, Black et al. (1978) showed that barbiturates decreased the size of infarcts, but increased mortality because of cardiac insufficiency. Controlled clinical studies of barbiturate treatment of a variety of disorders are in progress, but preliminary results are not encouraging.

Therapies of cerebral ischaemia:

A. Therapeutics proposed to increase the brain's ischaemic tolerance.

Metabolic depressants (e.g., hypothermia and barbiturates) have been investigated as a measure for protecting the brain from ischaemic damage. Hypothermia has been shown to reduce the cerebral metabolic rate of oxygen consumption (Smith and Wollman, 1972). Michenfelder (1977) studied the effect of prolonged hypothermia on MCA occlusion in monkeys and found that all treated monkeys died with infarction and cerebral oedema. He concluded that hypothermia induced an elevation in the blood viscosity, and that was responsible for the poor outcome. Furthermore, ischaemic brain damage is little influenced by this approach (Blöink et al. 1979).

B. Therapies proposed to improve blood flow.

1. Hypertension.

The loss of autoregulation in an ischaemic territory suggests that flow improvement should be possible through an increase in systemic blood pressure. Hope et al. (1977) observed an amelioration of neurological function by inducing hypertension, but there was evidence of an aggravation of brain oedema when blood pressure was increased (Fenske et al. 1978). Nevertheless, this treatment is now widely used for ischaemia after operation for a cerebral arterial aneurysm.

2. Haemodilution.

Infusion of dextran or albumin resulted in significant increases in blood flow (Sundt and Waltz, 1967; Crowell and Olsson, 1972; Blöink et al. 1979). However, the flow increase is only temporary (Sundt and Waltz, 1967) and haemodilution decreases the oxygen binding to the blood, and this may be the reason why haemodilution failed to prevent the development of cerebral oedema in ischaemic brain (Blöink et al. 1979).

3. Anti-oedema agents.

Osmotic agents (e.g., glycerol, sorbitol or mannitol) have been investigated as means of measuring the development of brain's oedema in ischaemia (Blöink et al. 1979; Little and O'Shaughnessy, 1979; Bremer et al. 1980). Little (1978) observed that mannitol improved neurological function and reduced infarct size in cats after MCA occlusion. Furthermore, in a double-blind clinical trial glycerol administration effectively

decreased oedema in acute cerebral ischaemia (Mathew, 1972; Lassen et al. 1976). However, improvement of flow is only temporary, and there is no influence on the electrolyte shifts associated with oedema (Hossmann, 1983). Treatment of oedema has been attempted using corticosteroids. Some authors observed certain improvement of oedema (Fenske et al. 1979; Bremer et al. 1980), but others failed to demonstrate an ameliorating effect (de la Torre and Surgeon, 1976; Little, 1978).

4. Vasodilators.

Cerebral vasodilation can be achieved either by hypercapnia or the administration of drugs capable of inducing cerebrovascular relaxation. Vasodilator therapy may have different mechanisms of action in the presence of focal ischaemic damage.

I. An improvement in CBF throughout the brain - including the lesion.

This can be achieved when the vasodilator agent produces elevation in total flow in the hemisphere. Thus, more blood will be delivered to the ischaemic areas of the brain, even if the latter remains within the same percent of total hemispheric blood flow.

II. Deleterious redistribution of the CBF.

In these circumstances, the total hemispheric blood flow may not be unchanged. However, vasodilation occurs preferentially in normal regions of the brain. This will result in these receiving an increased percent of hemispheric blood flow

compared to that of ischaemic regions of the brain in which lCBF will fall (steal phenomenon).

III. Beneficial redistribution of the CBF.

Again there may not be an alteration of total blood flow in the hemisphere, but preferential vasodilation occurs in the ischaemic territories and collateral supply. Thus, flow in this area increases at the expense of the rest of the hemisphere (Robin Hood syndrome).

The demonstration of Kety and Schmidt (1948) that 7% of CO_2 in inspired air increased cerebral blood flow led to studies of the possibility that CO_2 may contribute to the treatment of cerebrovascular disease. Furthermore, the capacity of pharmacological vasodilators to produce relaxation of cerebral vessels, and to improve the blood flow in normal and ischaemic brain, has been extensively studied (Lassen, 1959; Fazekas and Alman, 1965; Rosenblum, 1965; Regli et al. 1971a,b). Papaverine, aminophylline, hexobendine and numerous other vasodilating agents have been tested. However, few of these resulted in a consistent improvement of cerebral blood flow (Regli et al. 1971a; Berry et al. 1975; Blöink, 1979; Bartholini et al. 1980). Several authors have tested the possibility of increasing local cerebral perfusion in the presence of an ischaemic lesion, and the agents used included the potent physiological stimuli, hypercapnia (Shalit et al. 1967; Symon et al. 1971; Yamaguchi et al. 1971; Harrington et al. 1972) or hypocapnia (Battistini et al. 1971; Soloway et al. 1971; Harrington and DiChiro, 1973). Hypercapnia is assumed to improve the collateral circulation of the ischaemic tissues, and hypocapnia to induce vasoconstriction in the

non-ischaemic brain tissue, which might then improve the blood flow to the ischaemic territory by "reverse steal". The results were unsatisfactory; some authors observed that infarcts were smallest when CO_2 was kept as close as possible to normal value without any deviation toward hyper- or hypocapnia (Harrington and DiChiro, 1973).

It has been argued that when focal ischaemic damage is present, a drug that acts as a direct cerebral vasodilator could reduce the vascular resistance in non-ischaemic, healthy brain tissue, but not in the ischaemic territory because the vessels in the damaged region are already dilated. The consequences then would be an intracerebral steal with, at best, little improvement in the ischaemic territory and with the possibility of further reductions in CBF (Berry et al. 1975; Wilkins, 1980; Young et al. 1982, 1983; Hossmann, 1982).

Waltz (1971) wrote: "Present evidence provides no support for the use of vasodilators in the treatment of acute cerebral ischaemia". However, McHenry (1972), discussing the use of cerebral vasodilators in cerebral infarction, stated: "One can not accept the pessimism that there is no hope from more thorough evaluation and more aggressive management of the acute stroke".

The use of drugs to inhibit Ca^{++} entry during cell ischaemia and to induce vasodilatation of the cerebral vessels is a more recent approach. The extracellular Ca^{++} is approximately in the millimolar (10^{-3}) range, intracellular Ca^{++} is approximately 10^{-7}M (Bohr, 1973). This Ca^{++} concentration gradient is maintained probably by the cell membrane (Hagiwara and Byerly, 1981; Tsien, 1983) which has a low permeability to charged molecule and ions, and by an effective pump system that

maintains the Ca^{++} concentration at these low intracellular levels (Fabitor, 1983). A high level of permeability of cell membrane to calcium, and resulting dissipation of the Ca^{++} gradient, may lead to cell death (Fleckenstein et al. 1964; Hearse et al. 1977). Thus, massive influx of extracellular calcium into cell may initiate or represent a "Final Common Pathway" for cell death (Schanne et al. 1979; Farber, 1981). Calcium overloading may be involved in the pathophysiological processes of neuronal tissue damage (Harris et al. 1981; Hass, 1981). Thus, it has been shown that the extracellular calcium decreases during ischaemia, and spreading depression is presumed to be due to translocation of Ca^{++} into the intracellular compartments (Hansen, 1981; Harris et al. 1981; Siemkowicz and Hansen, 1981). An increase in the intracellular Ca^{++} is a prerequisite for cell death, as has been proposed for liver (Schanne et al. 1979), heart (Fleckenstein, 1977; Katz and Reuter, 1979; Nayler, 1983a,b) and cerebral tissue (Harris et al. 1981; Siesjö, 1981, 1984; Wieloch and Siesjö, 1982). It has been suggested that the influx of Ca^{++} into damaged tissue is due to disintegration of cell membranes, so that ions pass freely into the cell (Happel et al. 1981). This increase in Ca^{++} activity may arise both from the release of Ca^{++} from intracellular stores, or by influx from extracellular fluid (Peterson and Leblanc, 1976; Siesjö, 1984).

Several studies have found increases in cerebral Ca^{++} level in conditions of brain damage, e.g., in brains of mice exposed to cold stress (Korf et al. 1983), in the hippocampus during bicuculline or L-allylglycine induced seizures (Griffiths et al. 1982, 1983) and mechanically damaged spinal cord (Happel et al. 1981). There is extensive evidence that serotonin,

prostaglandins and noradrenaline may be the spasmogenic agents responsible for cerebral vessels constricting in the presence of blood in the subarachnoid space (Pennink et al. 1972; Allen et al. 1974; Starling et al. 1975; Boullin et al. 1976). All these substances act by increasing the concentration of intracellular free calcium, thereby promoting cerebrovascular smooth muscle cell contraction (Allen et al. 1974; Towart et al. 1981). The observation that vasoconstriction occurs in an area of focal ischaemia (Waltz and Sundt, 1967) raises the possibility that Ca^{++} antagonists might affect this, and therefore improve local CBF.

9. The Aims of the Studies.

In the present studies, the effects of intravenous administration of various concentrations of nimodipine on local CBF in normal, anaesthetised rats were examined, and focussed particularly on the extent to which either regional heterogeneity in the cerebral circulatory responses to nimodipine or changes in arterial blood pressure may have contributed to previous inconsistent data on the effect of calcium antagonists on cerebral tissue perfusion.

In an attempt to examine the functional significance of receptors for nimodipine present within the central nervous system (CNS), the effects of nimodipine on local cerebral glucose phosphorylation were studied in conscious, normal rats. Furthermore, in a parallel series of investigations, the effects of nimodipine on local CBF were examined, and compared and contrasted with the actions of nimodipine on local cerebral glucose utilisation.

In addition, a series of experiments were performed to determine if nimodipine reduced circulatory or pathological consequences of a well defined focal ischaemic lesion induced by occlusion of the middle cerebral artery.

CHAPTER II

MATERIALS AND METHODS

1. General

1.1 General surgical preparation.

A. Anaesthetised animals:

Experiments were performed with male Sprague-Dawley rats weighing between 300-470g. For the purpose of surgical preparation the animals were placed in a perspex box, when an anaesthetic gas mixture (70% nitrous oxide, 30% oxygen, containing 5% halothane) was given. Anaesthesia was maintained by means of a face mask by which a 1% halothane mixture was delivered. The animals were then tracheotomised, and mechanical ventilation (Sterling pump) with 0.5%halothane and N_2O - O_2 (70% - 30%) was used.

Small incisions (approximately 1 cm) were made in both groins. The femoral vessels were exposed and polyethylene catheters, 15 cm long and filled with heparinised saline solution (10 iu/ml), were introduced retrogradely into both femoral arteries and veins for continuous measurement of arterial blood pressure, withdrawal of arterial blood (for blood gas measurements), blood plasma glucose and blood sampling, and for administration of drug and tracer. The blood pressure was recorded by means of a Gould blood pressure transducer, blood gas measurements were performed using a Corning 168 pH/blood gas analyser, and the plasma glucose was measured using a Beckman glucose analyser.

At least 30 minutes were allowed to elapse before giving

the drug to achieve stability of arterial blood pressure, plasma glucose and blood gases. Normocapnia was maintained by adjustments in the tidal volume. Body temperature was maintained at 37 ± 0.5°C by keeping the animals on an automated heating box.

B. Conscious animals:

After inserting cannulae into both femoral arteries and veins, the cannulae and wounds were covered with local anaesthetic cream before closing the wounds with sutures. The wounds were covered with gauze pads, and plaster of paris bandage (Gypsona, width 7.5 cm) was placed around the lower abdomen, pelvis and upper third of thighs. The plaster and feet were taped to a lead weight to achieve immobilisation of the lower parts of the animal. Anaesthesia was then discontinued, and over two hours elapsed before starting the experiments. Body temperature was maintained at 37 ± 0.5°C by external heating.

1.2 Technique of middle cerebral artery occlusion in the rat.

The middle cerebral artery was occluded according to Tamura et al. (1981a). With the animal in the semi-prone position, left side uppermost, a 2 cm skin incision was made vertically between the orbit and the external auditory canal. The temporalis muscle was separated from the superior temporal line, and the tissue plane between the orbital fascia and the temporalis fascia was exposed to the temporal bone. The temporalis muscle was then retracted posteriorly and the orbital contents anteriorly, using curved self-retaining retractors. The infratemporal fossa was exposed and the mandibular nerve identified. A craniectomy was made using a dental drill 3 mm anterior and 1 mm lateral to foramen ovale. The dura was opened with a fine needle, and the middle cerebral artery identified running across the edge of the

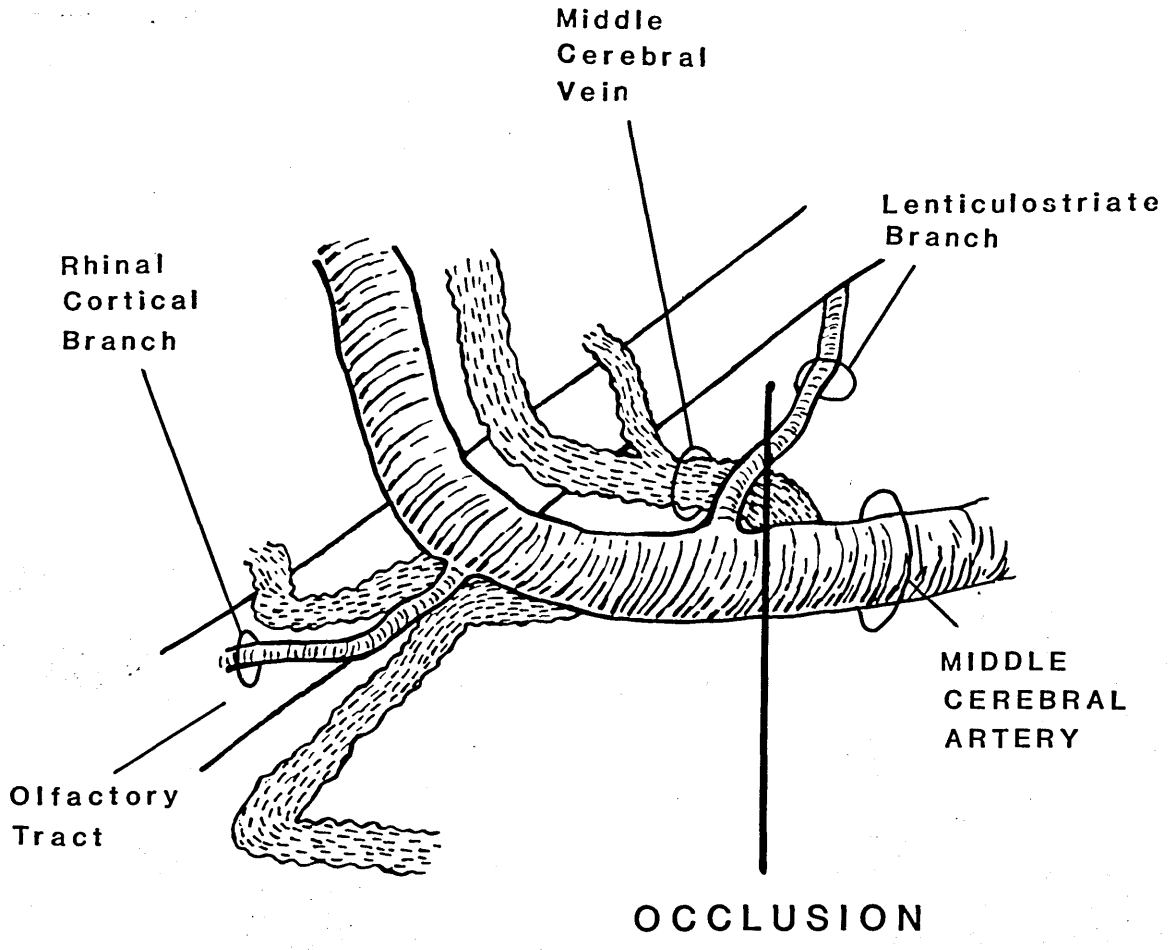
olfactory tract. The arachnoid was separated from the middle cerebral artery on each side, and a constant anterior branch running along the olfactory tract was found; a posterior branch, recurving proximally and giving deep lenticular striate branches, was also identified.

At the pre-designated time, the middle cerebral artery and these branches were occluded (Figure 3) using bipolar diathermy (microbipolar coagulator, Downs Surgical). The main trunk of the middle cerebral artery was then divided to ensure discontinuity.

1.3 Drugs and tracers.

Nimodipine solution was prepared freshly on the morning of each experiment. Because the breakdown of nimodipine is facilitated by white light, the drug was dispensed under sodium light; it was then added to a lutrol-glycerine-water mixture and heated gently with continuous stirring until fully dissolved. The drug, catheters and syringes for infusion were always covered with silver foil. Nimodipine was obtained from Bayer, U.K. The isotopes used were $[^{14}C]$-iodoantipyrine and $[^{14}C]$-2-deoxy-glucose (New England Nuclear, Boston, Massachusetts).

Figure 3.



Middle cerebral artery and its branches as seen under the microscope, with the site of occlusion. (From Shigeno et al. 1985).

2. Measurement of Local Cerebral Blood Flow

Local cerebral blood flow was measured with the quantitative autoradiographic technique with ^{14}C-iodoantipyrine as a tracer, described by Sakurada et al. (1978).

1. Theory.

Kety and Schmidt (1948) demonstrated that Fick's principle can be utilised to measure blood flow. Fick's principle stated that the quantity of a substance taken up by an organ in a unit time equals the total amount of the substance brought to it by the venous blood for the same period. The equation derived by Kety and Schmidt (1948) and Kety (1951, 1960) for calculating local blood flow is based mainly on balance at the capillary level.

$$\frac{dCi}{dt} = F(Ca-Cv) \qquad (1)$$

where Ci is the tissue concentration, F the blood flow per gram of tissue and Ca and Cv the arterial and venous concentration of the tracer, respectively. Provided instantaneous diffusion equilibrium occurs between blood and tissue, the tissue and blood concentrations of the tracer are related through the partition coefficient (λ), i.e.

$$Cv = Ci/\lambda \qquad (2)$$

Inserting this into equation (1) gives a first order differential equation that Kety solved for condition of Ci (0) to get

$$Ci(T) = \lambda K \; Ca_e - K(T-t) \; dt \qquad (3)$$

where Ci(T) equals the tissue concentration of the chemically inert diffusible tracer in a homogeneous tissue at a given time, T, after the introduction of the tracer into the circulation;

λ equals the tissue; blood partition coefficient; Ca is the concentration of the tracer in the arterial blood; t equals the variable of time; and K is the constant that incorporates within it the rate of blood flow in tissue. The constant K is defined by Kety (1951) as follows:

$$K = mf/w\ \lambda \qquad (4)$$

where f/w equals the blood flow per unit mean of tissue and m equals a constant between 0 and 1 that represents the extent to which diffusion equilibrium between blood and tissue is achieved during passage from arterial to venous end of the capillary. In the absence of diffusion limitation or arteriovenous shunt, m=1, and becomes inconsequential in the relationship between K and the perfusion rate of the tissue. The rate of blood flow can then be determined from the value for K calculated by the equation (3) from measured values of the tracer concentration in the tissue at a given time following the onset of circulation of the tracer, the history of arterial concentration of the tracer, and the tissue:blood partition coefficient.

2. Practice.

Rats were prepared according to the experimental design, and local cerebral blood flow measurement was performed using the diffusible tracer ^{14}C-iodoantipyrine (50 μCi in 1.5 ml saline). The isotope was infused intravenously over a 30 second period on a "ramp" schedule, i.e., the rate of infusion was increased progressively so that the arterial tracer concentration would be highest at the end of the experiment. Thus, the possibility of reaching a steady-state arterial tracer concentration can be avoided, and brain regions with high or low blood flow can be distinguished. During administration

of the tracer, arterial blood was allowed to drip freely from a partially occluded catheter in the femoral artery, and 15-18 samples of blood were taken separately on pre-weighed filter discs. The animals were sacrificed and the brains processed for quantitative autoradiography.

2.1 Liquid scintillation analysis.

The filter discs were quickly and tightly capped in scintillation vials to prevent evaporation, and re-weighed. From the assumed specific gravity of whole blood (1.05) (Sakurada et al. 1978) and the weight difference, the sample volume was calculated. Hydrogen peroxide (0.4 ml) and water (1 ml) were added to bleach the blood; thus, the possibility of colour quenching could be reduced. Vials were left at room temperature for at least 2-3 hours to allow completion of bleaching, and 10 mls of liquid scintillant (Picofluor) were then added. Twenty-four hours were allowed to elapse before liquid scintillation analysis. Each sample was counted for four minutes and the raw count data were converted to disintegrations per minute, using the external standard channels ratio method (Peng, 1977) together with a standard quench correction calibration curve.

2.2 Preparation of autoradiograms from brain sections.

At the end of infusion of the isotope, the animal was decapitated and the brain was quickly dissected. The dorsal cranium was removed, the dura was divided vertically from the olfactory lobe to the medulla oblongata, and then reflected. The whole brain was removed and frozen in isopentane, pre-cooled to -45°C. Decapitation and freezing of the brain were performed within 2-3 minutes to minimise the loss of resolution due to the

isotope diffusion. The frozen brain was embedded in tissue - Tek II (R.A. Lamb, London, U.K.). Brain sections (20 microns thick) were cut from the medulla oblongata to the prefrontal cortex in a cryostat (Bright, U.K.) at -22°C, and three in every 20 sections were mounted on glass cover slips and dried on a hotplate (60°C). The cover slips were fixed into thin cardboard and (together with a set of plastic standard (^{14}C) methylmethacrylate of known ^{14}C concentration (44-1475 nCi/g) which had been precalibrated for 20 um brain section) were applied to X-ray film (Kodak SB-5) for seven days in light-tight cassettes.

2.3 Quantitative densitometric image analysis.

2.3.1 Standard measurement.

Analysis of the resultant images on the X-ray film was performed using a computer-based densitometer (Quantimet 720, Cambridge Instruments), with reference to the precalibrated standards. For each region of the brain, twelve optical readings were made bilaterally on six consecutive sections in which the structure could be anatomically defined by reference to stereotactic atlases (Zeman and Innes, 1963; Konig and Klippel, 1963), and the mean optical density of these 12 measurements was used to calculate ^{14}C tissue concentrations. The areas of measurement varied from 0.25 mm^2 for large homogeneous regions of the CNS, such as hypothalamus and caudate nucleus, to 0.02 mm^2 for smaller brain areas such as substantia nigra (pars compacta) and hippocampus molecular layer. The size of the frame was maintained constant for the same region in different animals.

2.3.2 Frequency distribution analysis of the cerebral blood flow following MCA occlusion.

The use of conventional analysis of CBF (see 2.3.1) in the ischaemic model, employing the ^{14}C-iodoantipyrine autoradiographic technique, has been studied previously (Tamura et al. 1981b; Mohamed et al. 1983; Smith et al. 1983). In this method, selected neuroanatomically defined areas were measured. This approach may not fully assess the extent of changes in local CBF within or outside the territory of ischaemic cellular lesion. Therefore, frequency distribution of the CBF level was analysed in some experiments in this thesis (see Effect of nimodipine, 1 μg kg^{-1}min^{-1}, on the CBF with MCA occlusion - page 122) in an effort to provide more reliable information about the CBF level in the centre and periphery of the ischaemic lesion. In this method of analysis, the optical densities corresponding to particular CBF values were calculated by reference to the pre-calibrated standards on the film and the history of arterial tracer concentration. The distribution of blood flow was studied for the auditory cortex, parietal cortex, sensory-motor cortex and the caudate nucleus, ipsilateral and contralateral to the lesion. This was achieved by delineating the area examined (taken from three sections of autoradiograms of equal thickness) on the densitometer screen, using the image analyser and the densitometer function. The total delineated area was expressed by a picture point number obtained on the densitometer screen. Selected CBF levels at intervals of 25 ml 100g^{-1}min^{-1} were studied by dialling up the optical density which corresponded to cumulative flow bins (0-25, 50-75, ... 175-200). The area shown on the densitometer screen is represented by a picture point which has been recorded for each flow bin. The size of

area was then expressed as a percentage of total area. As an example, the following steps were taken to measure the CBF in the ipsilateral auditory cortex in a vehicle administration after MCA occlusion.

Firstly, the arterial concentration of the tracer was taken from the liquid scintillation analyser, together with the weighed samples, and the corresponding recorded time was entered into a computer (Cromenco System 3). The optical density of the plastic ^{14}C standards was measured by the densitometer. The computer then works out the best fit between the optical density (OD) and the known ^{14}C concentration (see Table 10). Secondly, using a computer programme designed by Dr. W. Angerson, the calculation of the CBF was determined for the ^{14}C-iodoantipyrine method (Sakurada et al. 1978). By dialling various values for optical density we obtained the corresponding CBF values (25,50,75 .... 200 ml $100g^{-1}min^{-1}$) (see Table 11). Thirdly, the ipsilateral auditory cortex was then delineated by the electric bin and a picture point figure corresponding to the total area was recorded from the densitometer screen. The optical density matching the particular cumulative flow-bin was then dialled up, and the new picture point figure was recorded for each flow level. The size of the area was expressed as a percentage of total area (see Table 12).

The total area of the ipsilateral auditory cortex was 33664. Therefore, by this method we can analyse the extent of ischaemic damage within and outside the lesion. From Table 12 we can see that 30% of the total area of the ipsilateral auditory cortex had a CBF level equal or less than

25 ml $100g^{-1}min^{-1}$, and 95.4% of the total area had a CBF equal or less than 100 ml $100g^{-1}min^{-1}$.

TABLE 10.

THE RELATIONSHIP BETWEEN STANDARD OPTICAL DENSITIES AND THE CONCENTRATIONS OF THE ISOTOPES.

Standard O.D.	Standard Concentration (nCi/g)	
	True	Best Fit
0.042	44	43
0.073	70	70
0.159	179	181
0.212	271	270
0.298	450	444
0.330	509	515
0.446	793	792

TABLE 11.

THE RELATIONSHIP BETWEEN THE OPTICAL DENSITY VALUE AND THE CORRESPONDING LEVEL OF THE CBF.

O.D.	CBF (ml $100g^{-1}min^{-1}$)
0.124	25
0.203	50
0.262	75
0.308	100
0.350	125
0.385	150
0.418	175
0.447	200

TABLE 12.

THE RELATIONSHIP BETWEEN THE CBF LEVEL AND THE AREA PERCENT CHANGE OBTAINED BY DENSITOMETER.

CBF Level	Area (Picture-Point	Area % Change
0-25	23539	30
25-50	10930	67.5
50-75	3694	89
75-100	1543	95.4
100-125	317	99
125-150	59	99.8
150-175	0	100
175-200	0	100

3. Potential Errors Associated with Measurements of Local Cerebral Blood Flow.

In the present work the method, the performance and factors that influence the accuracy of CBF values obtained by the ^{14}C-iodoantipyrine autoradiographic technique were examined. These factors include:

3.1 The accuracy of estimation of kill-time.

The kill-time is the time measured from the end of the isotope infusion to the completion of decapitation.

3.2 The accuracy of the catheter lag-time correction.

The concentration of tracer in the cerebral arterial blood at any time is assumed to be equivalent to that in the abdominal aorta (i.e., at the catheter tip). The concentration of the tracer sampled from the catheter is delayed relative to that in the brain by the amount of time it takes the blood to flow through the catheter (lag-time). This lag-time correction, which varies from animal to animal, is determined from the flow rate (number of drops/min by volume/drop) and the volume of the catheter.

3.3 The accuracy of the estimation of the partition coefficient.

The partition coefficient (λ) is the ratio of the solubility of the diffusible tracer in the brain tissue to that in the blood.

3.4 Errors associated with measurements of optical density consistency.

3.5 Errors associated with the accuracy of the radioactivity counts of the tracer.

Description of the theoretical model and generation of data.

A computer (Cromenco System 3, with disc-driven microprocessor units) was used for deriving the CBF data and associated variable parameters.

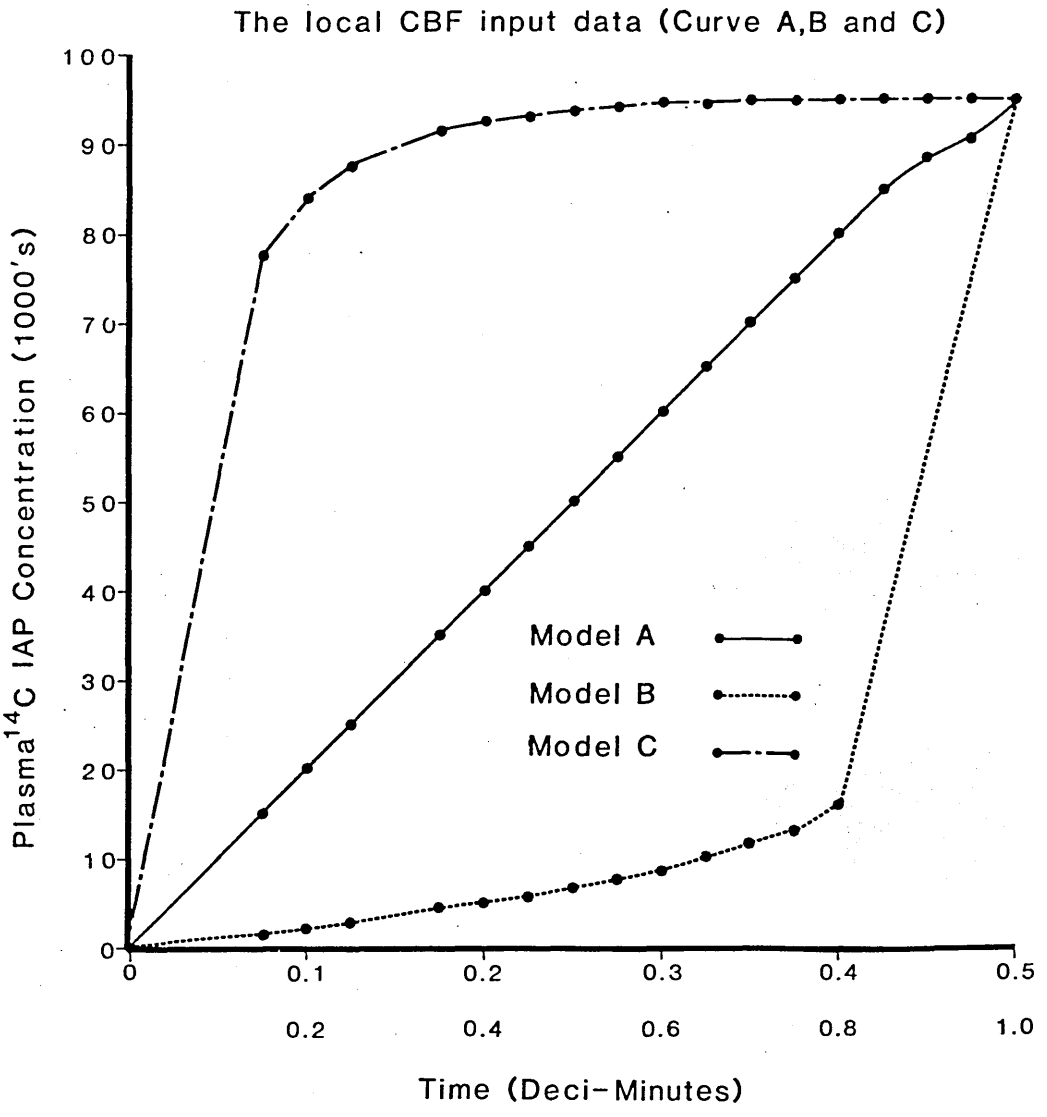
The determination of local CBF, using the quantitative autoradiographic technique (Sakurada et al. 1978) with ^{14}C-iodoantipyrine as tracer, is described in Chapter 2, Section 2. During the infusion of the tracer, 15-18 timed samples of arterial blood were collected in pre-weighed filter discs. By the end of the infusion period the animals were killed by decapitation, and the brains were quickly dissected and processed for quantitative autoradiography.

A typical arterial curve describing the concentration of the isotope and the time of the corresponding arterial sample is represented by Curve A (Figure 4). This curve was obtained by a "ramp" schedule of infusion, i.e., the rate of infusion was increased progressively. The other two extreme possibilities are shown in Curves B or C (Figure 4). In Curve C the infusion of the tracer was started at a very high concentration and kept approximately constant until the end of the infusion, whereas in Curve B the infusion was given in a very low concentration for a longer period and increased before the end of the infusion. These curves are plotted in Figure 4 with their respective ^{14}C-iodoantipyrine concentrations, together with the time of collection of each blood sample for 30 or 60 second periods of infusion. The number of drops, kill-time, catheter lag-time, the partition coefficient, the time for the infusion of the tracer and optical density standards are shown in Table 13.

TABLE 13. LOCAL CEREBRAL BLOOD FLOW INPUT DATA (CURVES A, B AND C).

Sample Number	Time (30 Sec)	Time (1 Min)	C^{14}-IAP Curve (A)	C^{14}-IAP Curve (B)	C^{14}-IAP Curve (C)	Sample Weight
1	0	0	0	0	0	0.025
2	0.075	0.15	15000	1500	77500	0.025
3	0.10	0.20	20000	2000	84000	0.025
4	0.125	0.25	25000	3000	87500	0.025
5	0.175	0.35	35000	4000	91500	0.025
6	0.20	0.40	40000	5000	92500	0.025
7	0.225	0.45	45000	5500	93000	0.025
8	0.25	0.50	50000	6500	93500	0.025
9	0.275	0.55	55000	7500	94000	0.025
10	0.30	0.60	60000	8500	94500	0.025
11	0.326	0.65	65000	10000	94500	0.025
12	0.350	0.70	70000	11500	95000	0.025
13	0.375	0.75	75000	13000	95000	0.025
14	0.40	0.80	80000	16000	95000	0.025
15	0.425	0.85	85000	20000	95000	0.025
16	0.45	0.90	88500	30000	95000	0.025
17	0.475	0.95	91500	55000	95000	0.025
18	0.50	1.00	95000	95000	95000	0.025

Figure 4.



The plasma $\left[^{14}C\right]$-iodoantipyrine concentration for Curves A, B and C infused at 30 secs or 1 min. (Time deci-min).

TABLE 14. BEST FIT FOR THE STANDARD OPTICAL DENSITIES FOR MEASURING THE CBF IN DIFFERENT INPUT DATA

Standard O.D.	Standard Concentration (nCi/ml)	
	True	Best Fit
0.444	44	44
0.070	70	70
0.179	179	179
0.271	271	271
0.450	450	450
0.509	509	509

These data are inserted into the computer to have the best fit for the standards of optical density (see Table 14), and the CBF values are obtained for each curve. For the sake of simplicity these curves, with their respective data, are called Models A, B or C.

The original data were stored in the computer, and a programme for the effect of changing one of the three parameters, i.e., kill-time, lag-time, and the partition coefficient (λ) on the estimated CBF value was designated by Dr. W. Angerson. For example, the cerebral blood flow (CBF) new values (2) in Model A at 30 sec infusion were as follows:-

Lag (min)		Kill-time (min)		Lambda		Flow (ml $100g^{-1}min^{-1}$)	
1	2	1	2	1	2	1	2
0.01	0.01	0.48	0.46	0.8	0.8	50	54
						100	109
						150	165
						200	277
						300	335
						350	393
						400	452

By this method the new blood flow values (2) are calculated when the new kill-time (0.46) is inserted.

The new values of the blood flow for Models A, B or C when the tracer is infused over 30 or 60 second periods are determined for the kill-time, catheter lag-time and partition coefficient (see Appendix I, Tables 1-17).

3.1 Errors associated with the accuracy of estimation of the kill-time.

Results

In Model A (30 sec infusion), an error of underestimation of the kill-time within a value of one second will increase the blood flow by 8-13%. Compared with a CBF base line ranging between 50 and 400 ml $100g^{-1}min^{-1}$, a two-second underestimation of the kill-time will produce elevation in the blood flow by 20-30%. By contrast, an overestimation of the kill-time by one or two seconds will produce reductions in the blood flow of 8-11% and 14-20%, respectively (see Figure 5, Appendix 1, Table 1). With a longer infusion time (60 sec), an underestimation by one or two seconds produced increases in blood flow of 4-9% and 10-20%, respectively. On the other hand, overestimation in the kill-time will produce reductions in flow levels of 4-9% and 8-16%, respectively (Figure 5).

In Model B (30 sec infusion), the blood flow levels increased by 34-71% and 72-161% with an underestimation of the kill-time by one and two seconds, respectively, and overestimation by one or two seconds will reduce the blood flow by 28-37% and 48-58%, respectively. With 60 sec infusion, an error of one or two seconds of underestimation of kill-time increased blood flow values by 20-43% and 40-100%, respectively. With overestimation by one or two seconds, the CBF levels reduced by 16-27% and 30-44%, respectively (see Figure 6).

In Model C (30 sec infusion), the flow levels increased by 4-5% and 10-16% with an underestimation in the kill-time by one or two seconds, respectively. Overestimation in the kill-time in the same magnitudes produced reductions in CBF values of

8-10% and 4-5%. In Model C (60 sec infusion), an underestimation or overestimation in the kill-time within a value of ± 1 or ± 2 seconds will produce a CBF level ranging between ± 2% to ± 10% (Figure 7).

3.2 Errors associated with the accuracy of the catheter lag-time estimation.

The catheter lag-time is dependent on the catheter flow in blood flow experiments. The lag-time is corrected to the rate of the catheter flow in many laboratories, and catheter flow (ml min^{-1}) has been measured in our laboratory. It was found that the lag-time relationship to the catheter flow rate is as follows:

Catheter flow (ml min^{-1})	Catheter lag-time (min)
Greater than 2	0.02
1-2	0.03
0.8-1	0.04
0.6-0.8	0.05

Results

The lag-time correction and relationship to CBF are shown in Figure 8. In Model A (30 sec infusion). a lag-time correction by 0.02, 0.03 and 0.04 compared to a base line lag-time of 0.01 reduced the blood flow by 4-6%, 8-10% and 10-15%, respectively. In Model A (60 sec infusion), the blood flow values decreased by 2-5%, 4-9% and 6-12%, respectively.

In Model B (30 sec infusion), a lag-time correction with the same magnitudes reduced the blood flow by 14-22%, 28-37% and 38-49%, respectively. With a longer period of infusion

(60 sec), the blood flow levels decreased by 8-15%, 16-27% and 22-36%. In Model C (30 or 60 sec infusion), the blood flow values reduced by 1-5%.

3.3 Errors associated with the accuracy of the estimation of the partition coefficient.

Knowledge of the partition coefficient (λ) is required for the quantitative measurement of CBF in all diffusible tracer techniques. This necessitates a special experimentation in which an equilibrium state must be attained for tracer concentrations in the blood and cerebral tissues, and then maintained over a sufficiently long period. The value of λ was reported by Sakurada et al. (1978) to be 0.8 in albino rats in which the tracer in the arterial blood was maintained at a constant concentration for at least two hours for blood:brain equilibrium.

In normal conditions, amounts of diffusible tracer which are contained in the arterial blood are redistributed between the brain and the blood according to respective solubilities. In case of a diffusible tracer, λ is thus the ratio of the solubility of the diffusible tracer in the brain tissue to that in the blood (Kety, 1951).

Results

The relationship between errors in the partition coefficient and CBF is shown in Figures 9-11.

In Model A (30 sec infusion), when the partition coefficient (λ) was reduced to 0.7 and 0.6, the blood flow values were increased by 2-22% and 4-60%, respectively. On the other hand, an elevation in the estimation of lambda by 0.09 and 1.0 decreases the flow levels by 2-11% and 2-18%. With a 60 sec infusion in

Model A, with lambda 0.7, the blood flow levels increased by 4-67% compared to CBF base line range between 50 and 400 ml $100g^{-1}min^{-1}$. When lambda was reduced to 0.6, the blood flow levels increased by 8-89% compared with the CBF base line of 50-200 ml $100g^{-1}min^{-1}$. When there was an elevation in the lambda to 0.9 and 1.0, the blood flow values decreased by 2-24% and 4-35%, respectively.

In Model B (30 or 60 sec infusion), the blood flow was increased by 0-7%, 2-17% and 2-10%, 4-25%, respectively, when underestimations of the partition coefficients were used at (0.8) by 0.7 and 0.6, respectively. In cases of overestimation of lambda to 0.9 and 1.0, the flows in Model B (30 or 60 sec infusion) diminished to 0-5%, 2-9% and 2-8%, 2-13%, respectively.

In Model C (30 sec infusion), flow levels varied between 50 and 350 ml $100g^{-1}min^{-1}$, and were increased by 2-56% when lambda decreased by 0.01. With further decrease in lambda to 0.6, flow range between 50 and 250 ml $100g^{-1}min^{-1}$ increased by 6-120%. However, the flow levels decreased by 2-20% and 2-29% when lambda increased by 0.01 and 0.02, respectively. In Model C (60 sec infusion), a base line flow ranging between 100 and 150 ml $100g^{-1}min^{-1}$ will increase by 14-35% when lambda is reduced to 0.7. Base line flow of 100 ml $100g^{-1}min^{-1}$ increased by 49% with value of lambda of 0.6. In the case of lambda increases 0.9 and 1.0, the flow reduced by 8-45% and 13-54%, respectively, compared with base line flow levels of 50-500 ml $100g^{-1}min^{-1}$.

3.4 Errors associated with measurements of optical density consistency.

The data output of the densitometer is the "total integrated density" of a feature or area of interest. This is the sum of the optical density contribution of each individual point within the detected region. For each brain region, 6 or 12 bilateral optical density readings can be measured by the quantitative densitometric analyser (see Chapter II, Section 2.3). The mean value and ± SEM of these readings are obtained. The SEM for each brain structure is usually accepted in our laboratory when it is ± 10% of the mean optical density, approximately.

Results

The influence of a SEM of ± 10% for optical density is shown in Figure 12 (see also Appendix II, Tables 18-23).

In Model A (30 sec infusion), a SEM of OD of + 10% produced increases in the levels of blood flow by 12-25%, whereas a SEM of -10% reduced blood flow values by 12-20%. In Model A (60 sec infusion), + 10% produced increases in the flow by 12-21% in the CBF range 50-150 ml $100g^{-1}min^{-1}$, and by 26-63% in the range 200-400 ml $100g^{-1}min^{-1}$. On the other hand, -10% decreased flow values by 12-17% with CBF in the range 50-150 ml $100g^{-1}min^{-1}$, and by 20-30% in the flow range 200-400 ml $100g^{-1}min^{-1}$.

In Model B (30 sec infusion), a SEM of ± 10% of the measured optical density increased the blood flow by 8-15%, and decreased by 12-14% compared with CBF range 50-400 ml $100g^{-1}min^{-1}$. In Model B (60 sec infusion), ± 10% produced elevations by 8-15%, and decreased by 12-14% in flow range 50-400 ml $100g^{-1}min^{-1}$.

In Model C (30 sec infusion), + 10% produced increases by

12-16% in the CBF values and -10% decreased the flow by 10-26% compared with the CBF levels of 50-400 ml $100g^{-1}min^{-1}$. In Model C (60 sec infusion), + 10% produced an increase in the flow by 20-67% in CBF range 50-200 ml $100g^{-1}min^{-1}$.

3.5 Errors associated with the accuracy of the radioactivity counts of the tracer.

Results

The ^{14}C-iodoantipyrine blood sample concentrations associated with errors produced by the liquid scintillation analyser within a value of -1%, -5% or -10% are shown in Figure 13a, b (see also Appendix III, Tables 25-30).

In Model A (30 sec infusion), an error of underestimation of radioactivity concentration of the ^{14}C-iodoantipyrine within a value of -1% increased the CBF by 2-4% in flow levels range 50-400 ml $100g^{-1}min^{-1}$, whereas underestimation of -5% in the radioactivity produced an increase in flow by 8-14% compared with CBF range 50-400 ml $100g^{-1}min^{-1}$. When the liquid scintillation analyser failed to detect the radioactivity level by -10%, the blood flow elevated by 14-31%. In Model A (60 sec infusion), -1%, -5% and -10% in the radioactivity of the isotope increased the level of CBF by 2-5%, 6-27% and 14-77%, respectively.

In Model B (30 or 60 sec infusion), the tendency of increasing the level of CBF with underestimation of the tracer counts is generally the same in both 30 and 60 second periods of infusion.

In Model C (30 sec infusion), underestimation of -1%, -5% or -10% increased the CBF by 2-4%, 8-24% and 14-78% compared to CBF range 50-400 ml $100g^{-1}min^{-1}$. Underestimation of the isotope

concentration in Model C (60 sec infusion) by -1% increased the flow levels by 2-20% compared to CBF range 50-400 ml $100g^{-1}min^{-1}$, and a decrease in the tracer counts by -5% produced an increase in CBF levels by 12-121% compared to CBF range 50-300 ml $100g^{-1}$ min^{-1}. Further reduction in the isotope concentration by -10% increased the CBF levels by 20-85% compared to CBF range 50-200 ml $100g^{-1}min^{-1}$.

Summary of the potential errors associated with measurements of local CBF.

Errors of the kill-time.

It is important to have the accurate kill-time for the blood flow experiments. Errors in estimating that are more likely to add a variable factor. The sacrificing of large animals (baboons) is technically more difficult than small animals (rats). Investigators may use Kcl to arrest the circulation. The possibility of having errors of either over- or underestimation of the accurate kill-time is greater in these circumstances. It is more accurate to have a blood data curve situated between Models A and C to minimise the influence of inaccuracy of kill-time estimation of blood flow data.

Errors of the lag-time.

Models A and C tolerate errors associated with lag-time correction better than Model B. There were no significant differences in the errors in models conducted over 30 or 60 sec infusions.

Errors associated with the partition coefficient.

It is necessary to show concern in quantitative measurements of blood flow in pathological conditions (cerebral ischaemia, cerebral neoplasm, cerebral oedema, etc.), where λ may be changed. The measurements of blood flow in the present studies (see experimental design) were made 30 min after middle cerebral artery occlusion. Eklof et al. (1974) suggested that there were no major changes in the partition coefficient, particularly in ischaemic areas with low blood flow. Thus, these changes in λ make relatively minor differences to the calculated levels of CBF.

Errors of the optical density measurement.

It is also important to avoid the problem of thick and thin sections of the brain when measuring the optical density of the CNS regions. The calibration and stability of the Quantimet densitometer is also important, to avoid variation in optical densities.

Errors of the ^{14}C-IAP estimation.

Inaccurate estimation of the ^{14}C-iodoantipyrine concentration by liquid scintillation analysis is an important factor associated with the variability of CBF data. Underestimation of the isotope's radioactivity concentration produces increases in CBF values in all types of blood flow experiments. It is obvious from the results that a 30 second period of infusion tolerates errors produced by the liquid scintillation analysis better than that of 60 seconds (see Figure 13a and b).

The bleaching of blood samples containing the tracer (see Chapter II, Section 2.2) is an important procedure to reduce the

possibility of colour quenching. The vials of the blood samples should be allowed to remain at room temperature for at least two hours for bleaching to proceed. it is equally important to check the calibration of the liquid scintillation analyser, together with monitoring the accuracy of the measurement, by using two vials (usually 7 and 10) from each blood flow experiment subjected to the internal standard method.

In the experimental work in this thesis, the use of "ramp" schedule infusion over 30 sec resulting in having blood flow data resembling Curve A. The maximum errors related to under- or overestimation of the kill-time in Model A (30 sec infusion) were elevation in the CBF by 13% (underestimation of the kill-time by 1 sec), and reductions in the blood flow levels by 11% (overestimation of the kill-time by 1 sec) compared with a CBF base line of 400 ml $100g^{-1}min^{-1}$.

As shown in the results, Model A (30 sec infusion) minimised the influence of inaccurate estimation of the kill-time on the CBF levels. Furthermore, the use of small animals (rats) made it technically possible to have accurate estimation of the kill-time.

In Model A (30 sec infusion), a lag-time correction by 0.02 compared to a base line lag-time of 0.01 reduced the blood flow by 4-6%. In the majority of the blood flow experiments conducted in the present studies, the catheter flow was greater than 2 ml min^{-1}; therefore, the catheter lag-time was 0.02 (min).

During the measurements of the optical density of the brain regions, the problem of the thick and thin sections was avoided and careful calibration of the Quantimet was performed. The use of Model A (30 sec infusion) in this thesis takes into account both biological variability and experimental errors.

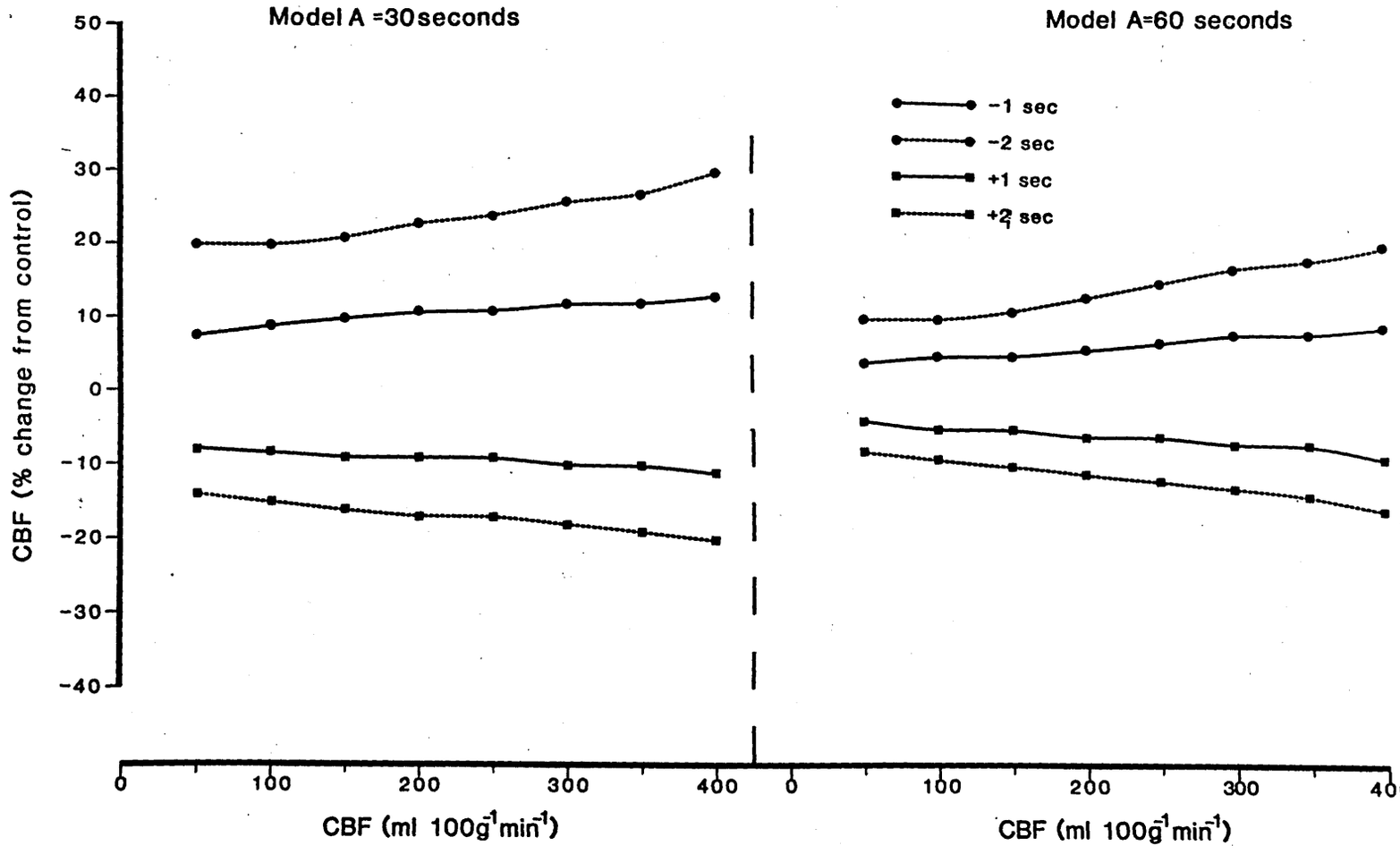
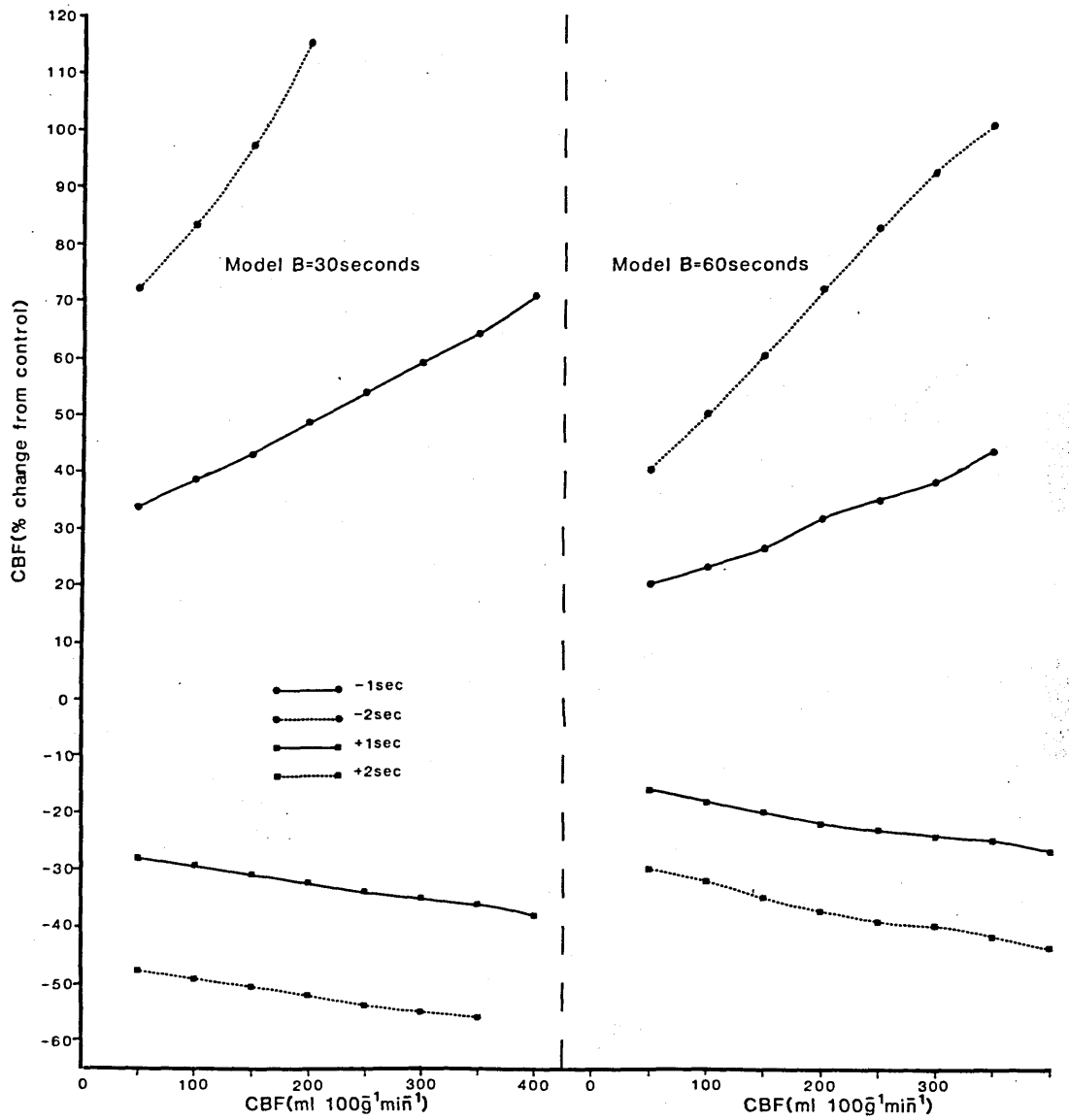


Figure 5. Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of kill-time within a value of $\pm$ 2 sec.

Figure 6.



Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of kill-time within a value of $\pm$ 2 sec.

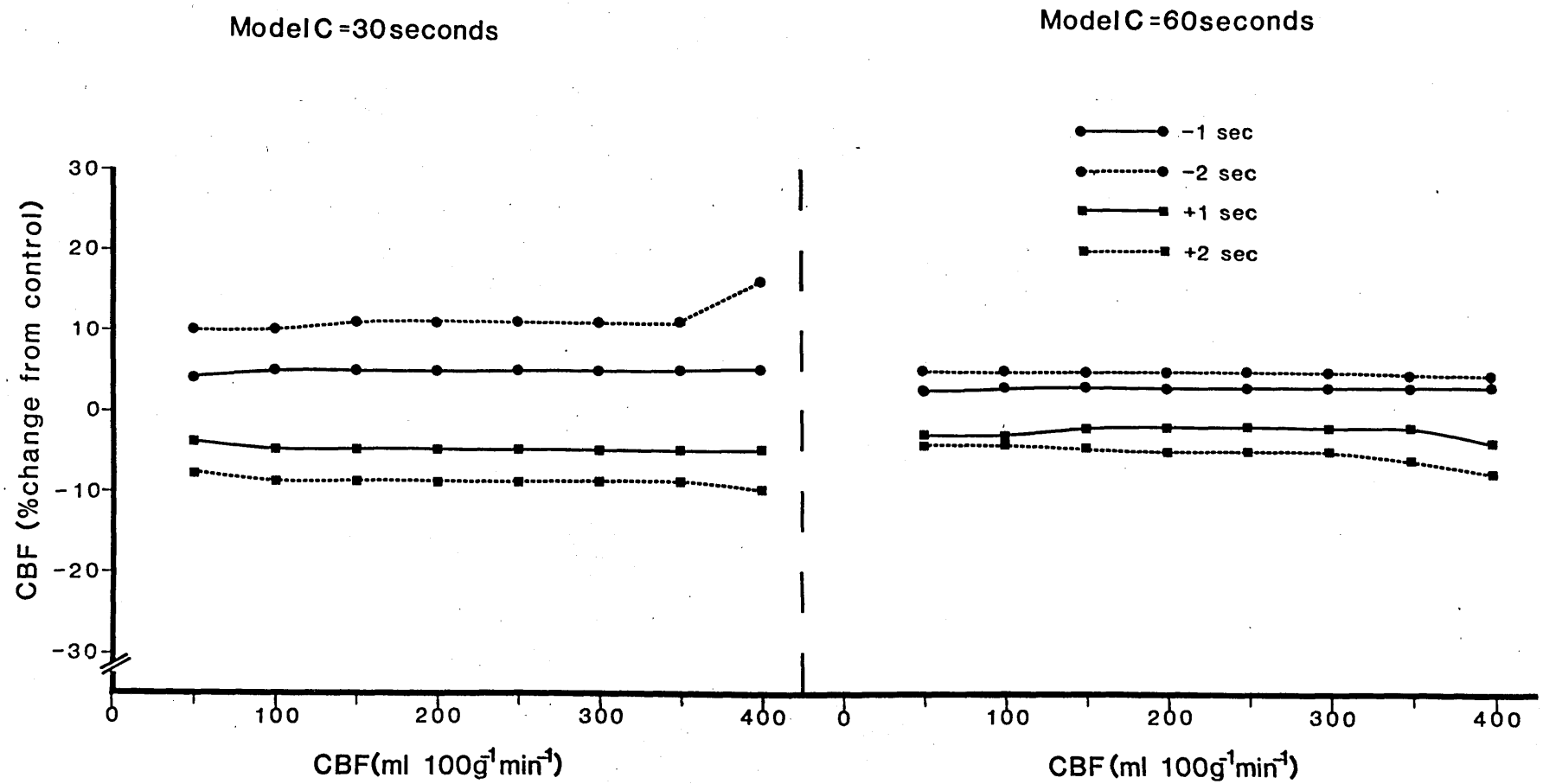


Figure 7. Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of kill-time within a value of $\pm$ 2 sec.

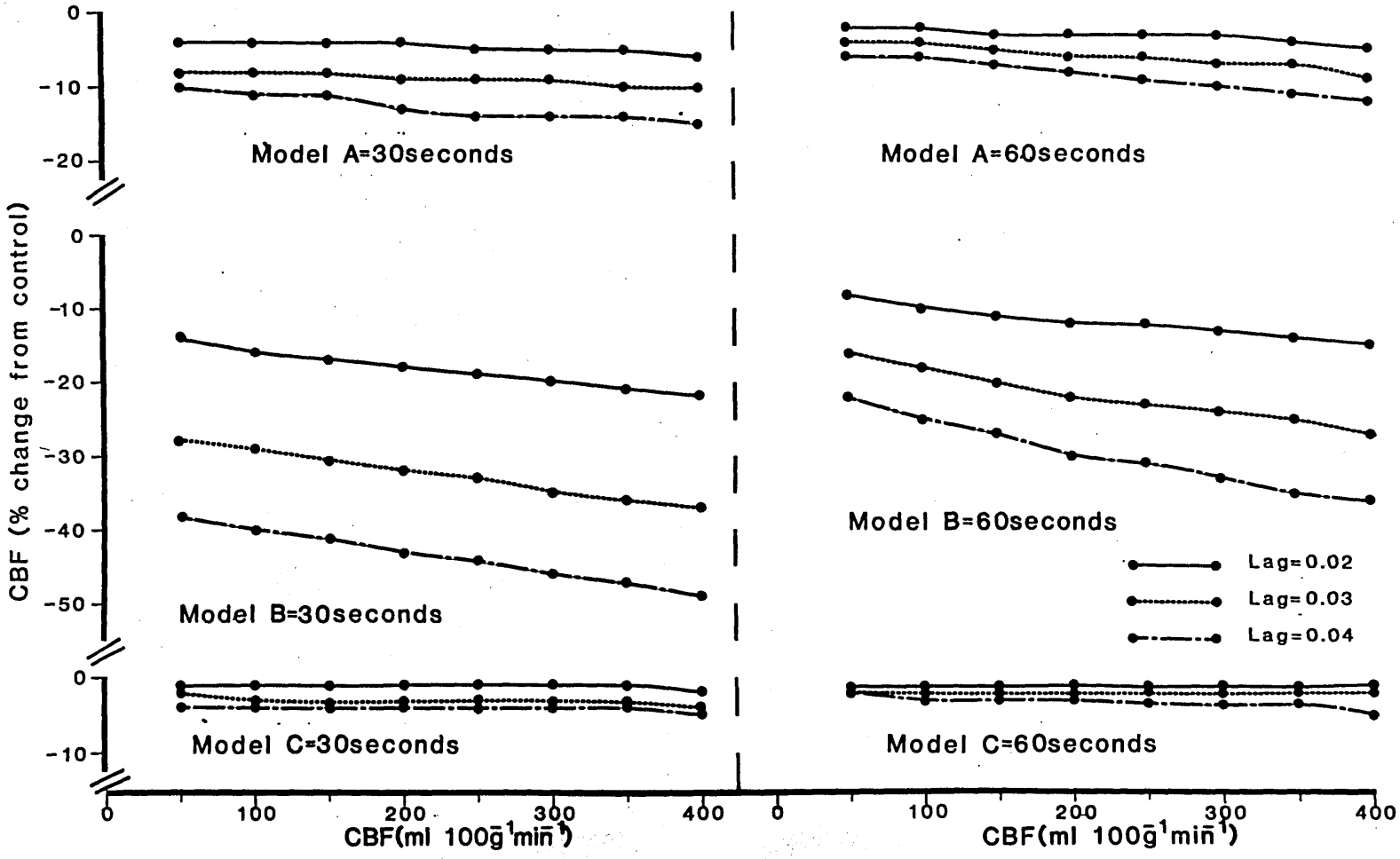


Figure 8. Variability of CBF (ml $100g^{-1}min^{-1}$) related to lag-time correction within a value of + 0.01, + 0.02 or + 0.03, compared to control lag-time (0.01).

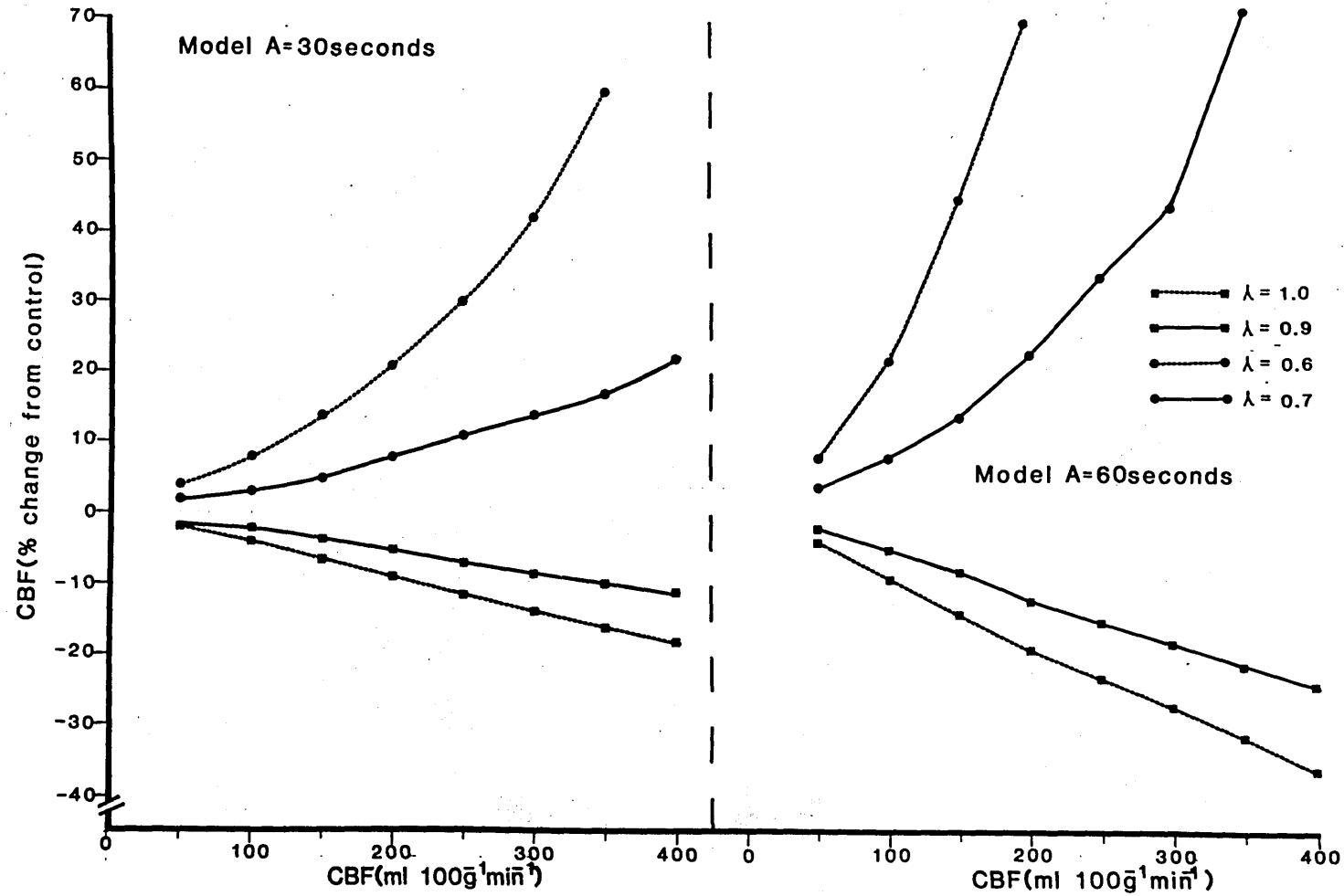


Figure 9. Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of the partition coefficient within a value of $\pm$ 0.1 or $\pm$ 0.02, compared with a control value of 0.8.

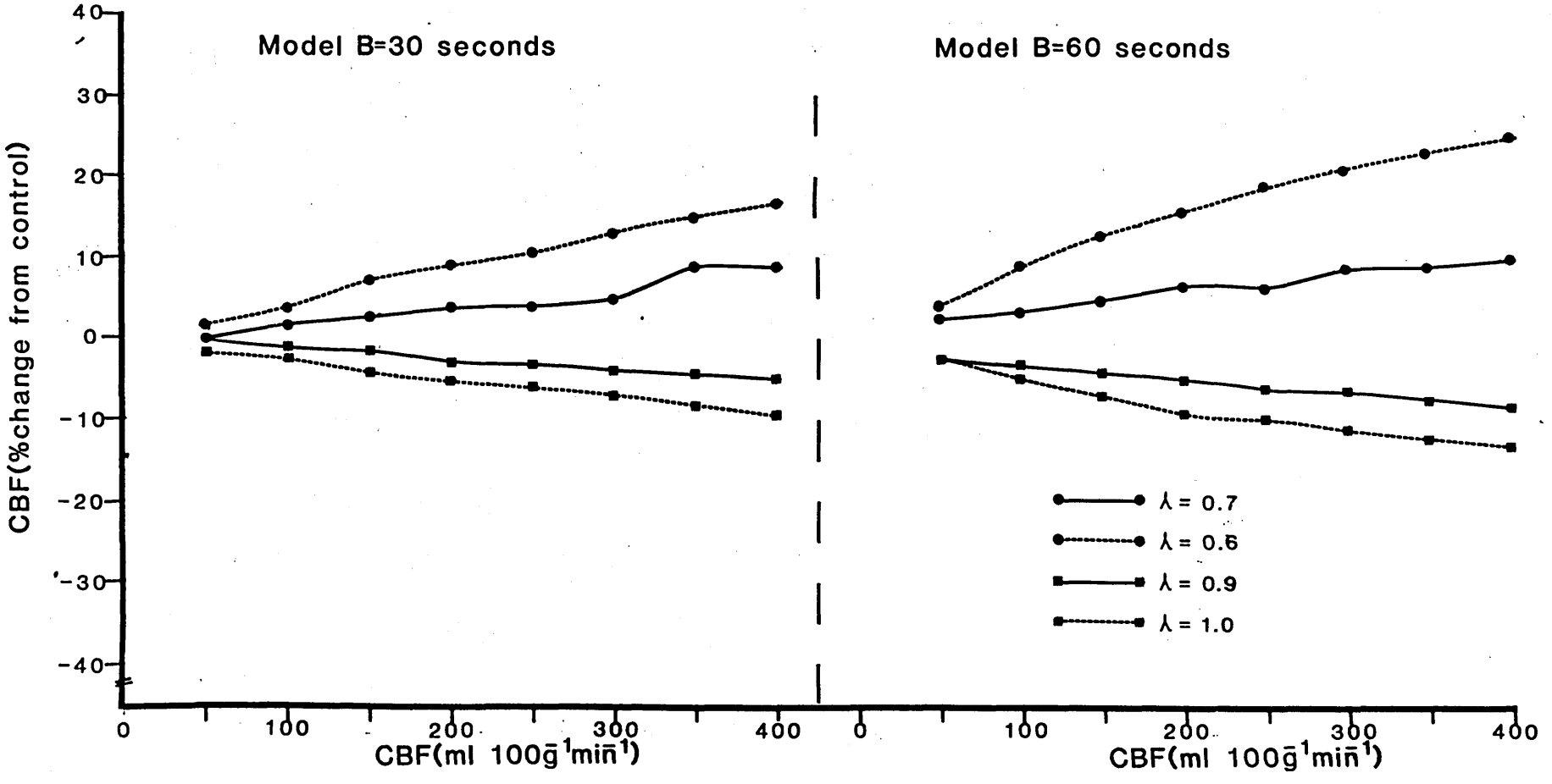


Figure 10. Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of the partition coefficient within a value of $\pm$ 0.1 or 0.02, compared with a control value of 0.8.

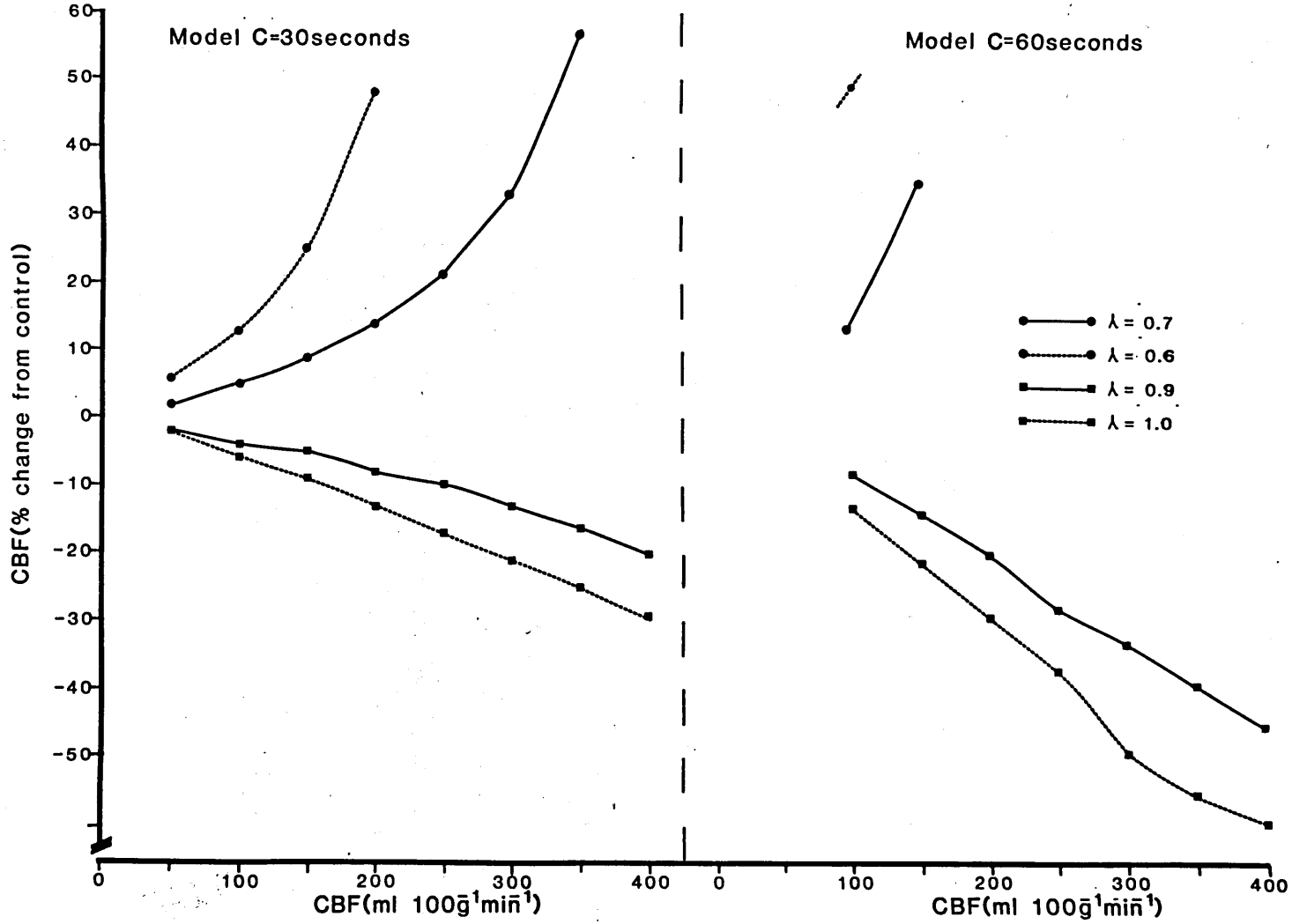


Figure 11. Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of the partition coefficient within a value of $\pm$ 0.1 or 0.02, compared with a control value of 0.8.

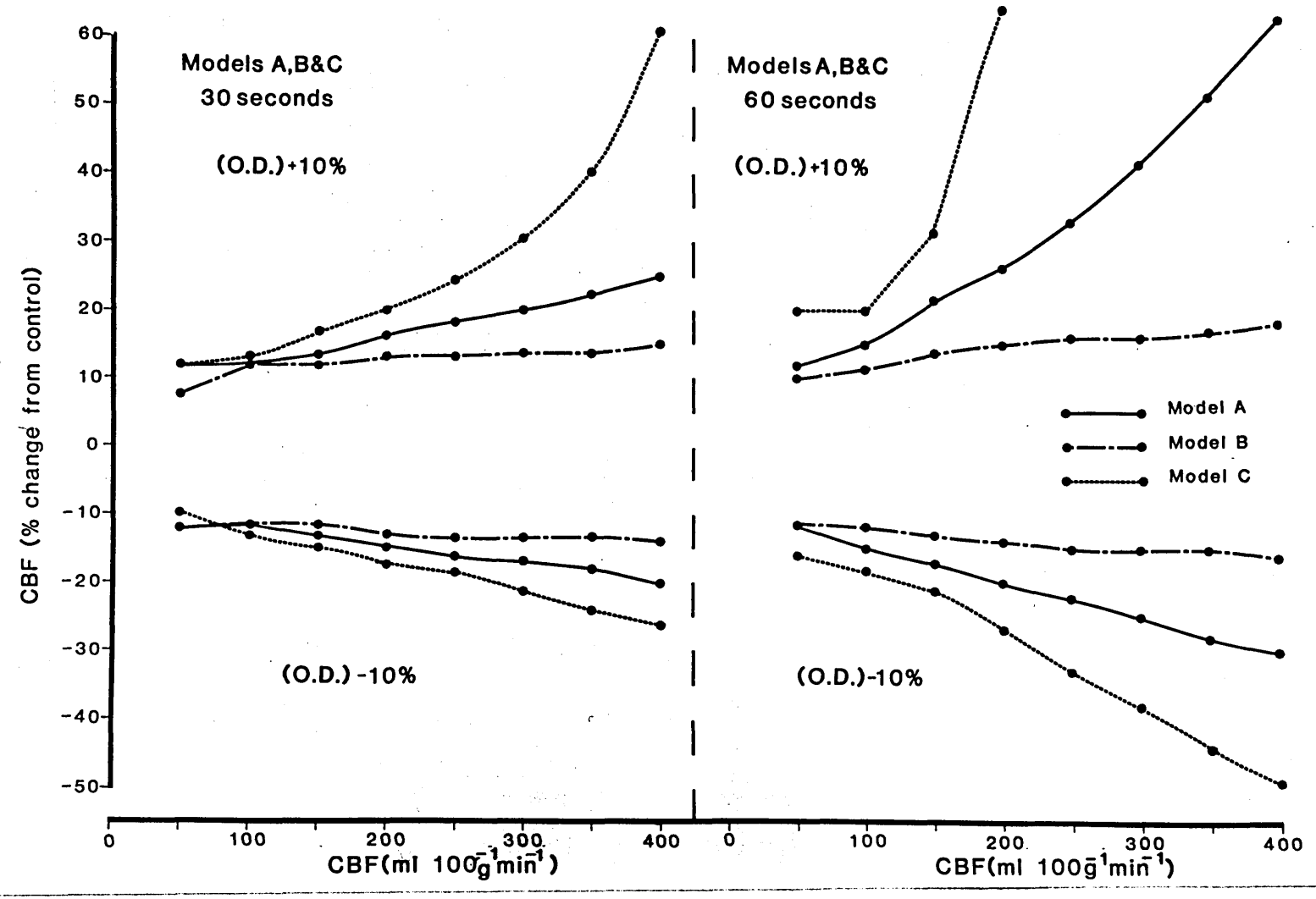


Figure 12. Variability of CBF levels and relationship to optical density measurement within a value of ± 10%.

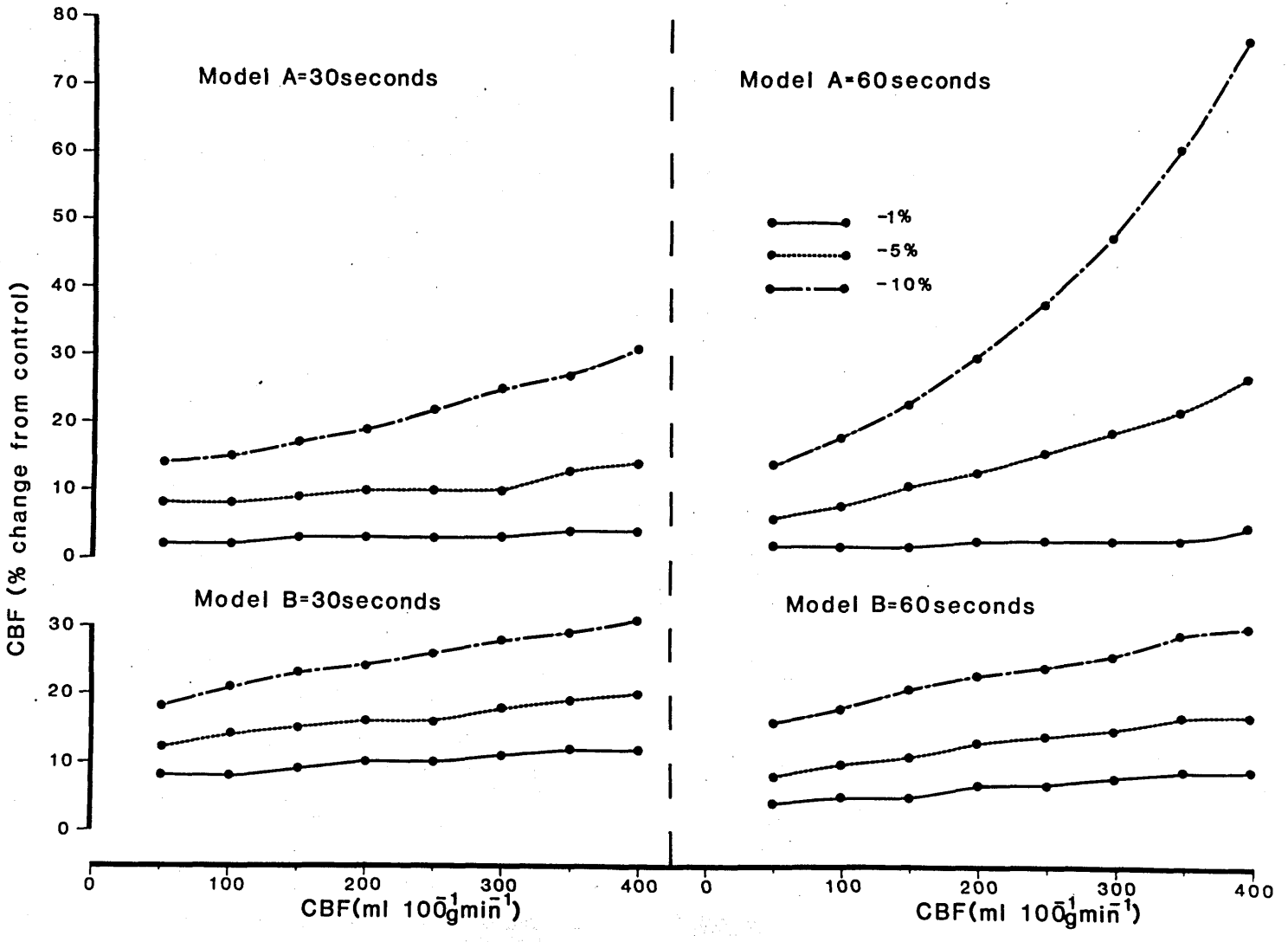


Figure 13a. Variability of CBF (ml $100g^{-1}min^{-1}$) and $[^{14}C]$-iodoantipyrine underestimation within a value of -1%, -5% and -10%.

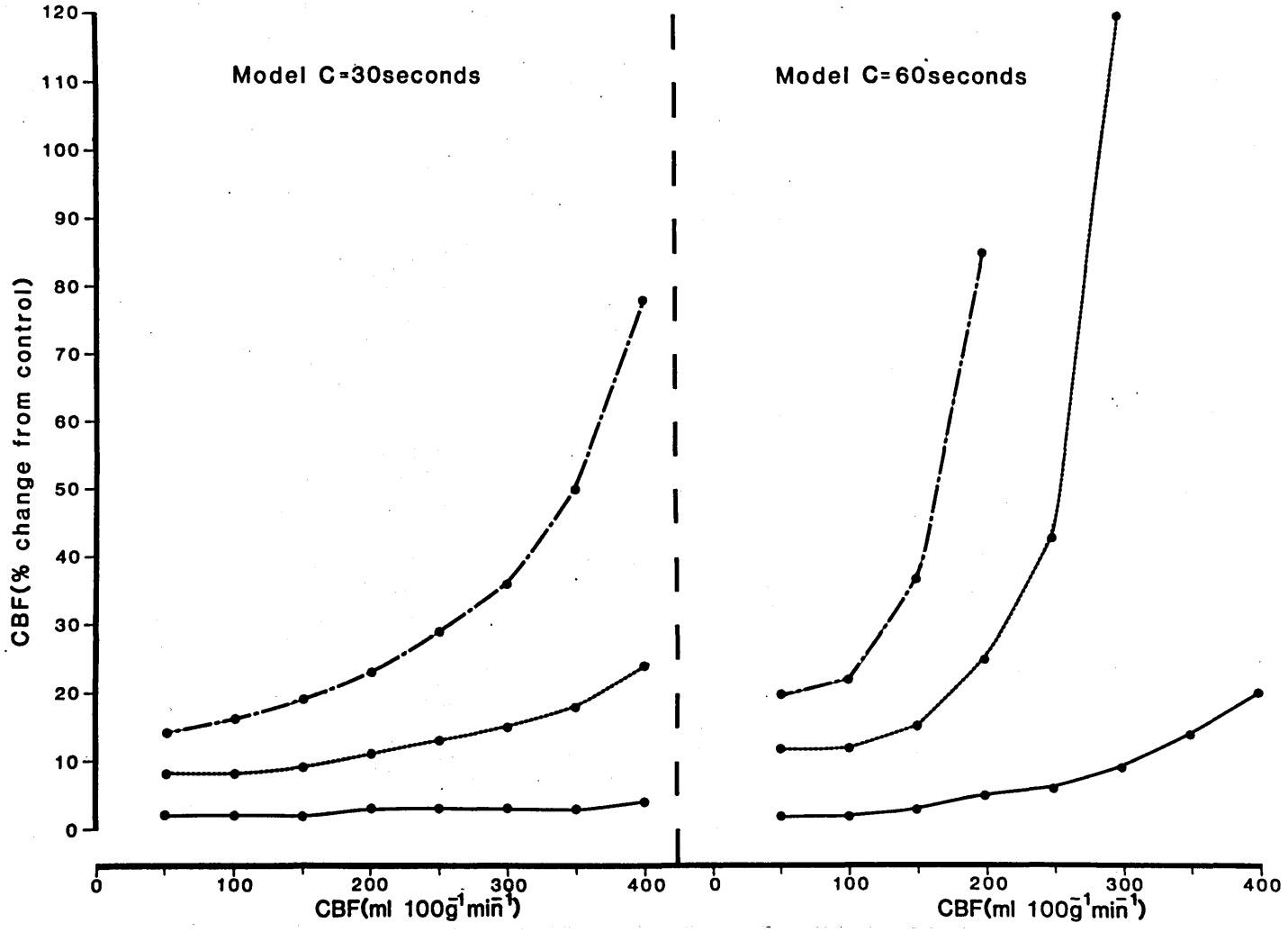


Figure 13b. Variability of CBF (ml $100g^{-1}min^{-1}$) and $[^{14}C]$-iodoantipyrine underestimation within a value of -1%, -5% and -10%.

4. Measurement of Local Cerebral Glucose Utilisation

Local cerebral glucose utilisation was measured using the ^{14}C-2-deoxyglucose technique (Sokoloff et al. 1977).

4.1 Theory.

The knowledge about the brain energy metabolism has been obtained from the estimation of cerebral metabolic rate of oxygen, using the nitrous oxide technique (Kety and Schmidt, 1948) and its modifications (Scheinberg and Stead, 1949; Lassen and Munck, 1955; Eklof et al. 1973; Gjedde et al. 1975) which measures the average rates of energy metabolism in the whole brain.

Oxygen and glucose use are the normal substrates of cerebral energy metabolism. Under normal circumstances, the brain uses glucose primarily to fuel many biochemical reactions necessary for cerebral function (Gibbs et al. 1942; Kety, 1948; Hawkins et al. 1974; Sokoloff et al. 1977; Siesjö, 1978).

Measurements of the oxygen uptake or glucose use by the brain provide an index of neuronal functional activity of the brain (Kennedy et al. 1975; Sokoloff et al. 1977). Sokoloff et al. (1977) have established a method which measures the rates of energy metabolism in specific discrete regions of the brain in normal and altered conditions of the functional activity by employing the ^{14}C-2-deoxyglucose autoradiographic technique. Thus, the limitations of the ^{14}C-glucose method (Hawkins et al. 1974) can be avoided.

The only difference between 2-deoxyglucose and glucose is in the hydrogen atom attached to the second carbon in place of a hydroxyl group. Sokoloff's technique (1977) for the

measurement of cerebral glucose utilisation was based on taking advantage of the absence of this hydroxyl group from the molecular structures of 2-deoxyglucose. Both sugars have been shown to share the hexose transport system; transport system between the blood and brain (Oldendorf, 1971; Horton et al. 1973). In cerebral tissue, both sugars are substances for the hexokinase reaction, in which they are phosphorylated to 2-deoxyglucose-6-phosphate and glucose-6-phosphate (Sols and Crane, 1954). 2-Deoxyglucose-6-phosphate, unlike glucose-6-phosphate, is not a substrate for glucose isomerase or glucose-6-phosphate dehydrogenase, and its catabolism ceases at this point (Sols and Crane, 1954; Wick et al. 1957; Tower, 1958; Horton et al. 1973). Deoxyglucose-6-phosphate can be broken down to deoxyglucose by glucose-6-phosphatase (the action which results in the hydrolysis of deoxyglucose-6-phosphate to deoxyglucose). In cerebral tissue, the activity of this enzyme is very low (Raggi et al. 1960; Prasannan and Subrahmanyam, 1968).

The mathematical model for the quantitative determination of regional glucose utilisation (Figure 14) was developed on the assumption that the ^{14}C-deoxyglucose phosphate is essentially trapped in the cerebral tissue, and it is neither metabolised further at an appreciable rate nor lost from the site of phosphorylation (Sokoloff et al. 1977). Some investigators (Hawkins and Miller, 1978; Hawkins, 1980; Sacks et al. 1983) questioned the basic premise of the procedure, and doubted if the loss of deoxyglucose phosphate could be ignored. However, no convincing evidence against the method emerged from these investigations. Subsequently, a number of detailed reviews have been published in which the features of this

particular method were emphasised (Sokoloff, 1978, 1979, 1981a,b,c, 1982; Sokoloff et al. 1983).

The main limitations and conditions of the ^{14}C-2-deoxy-glucose technique are:-

a) The value of the lumped constant and the kinetic constants must be known for the region and species being investigated.

b) The cerebral glucose utilisation and the plasma glucose concentrations remain constant throughout the period of measurement.

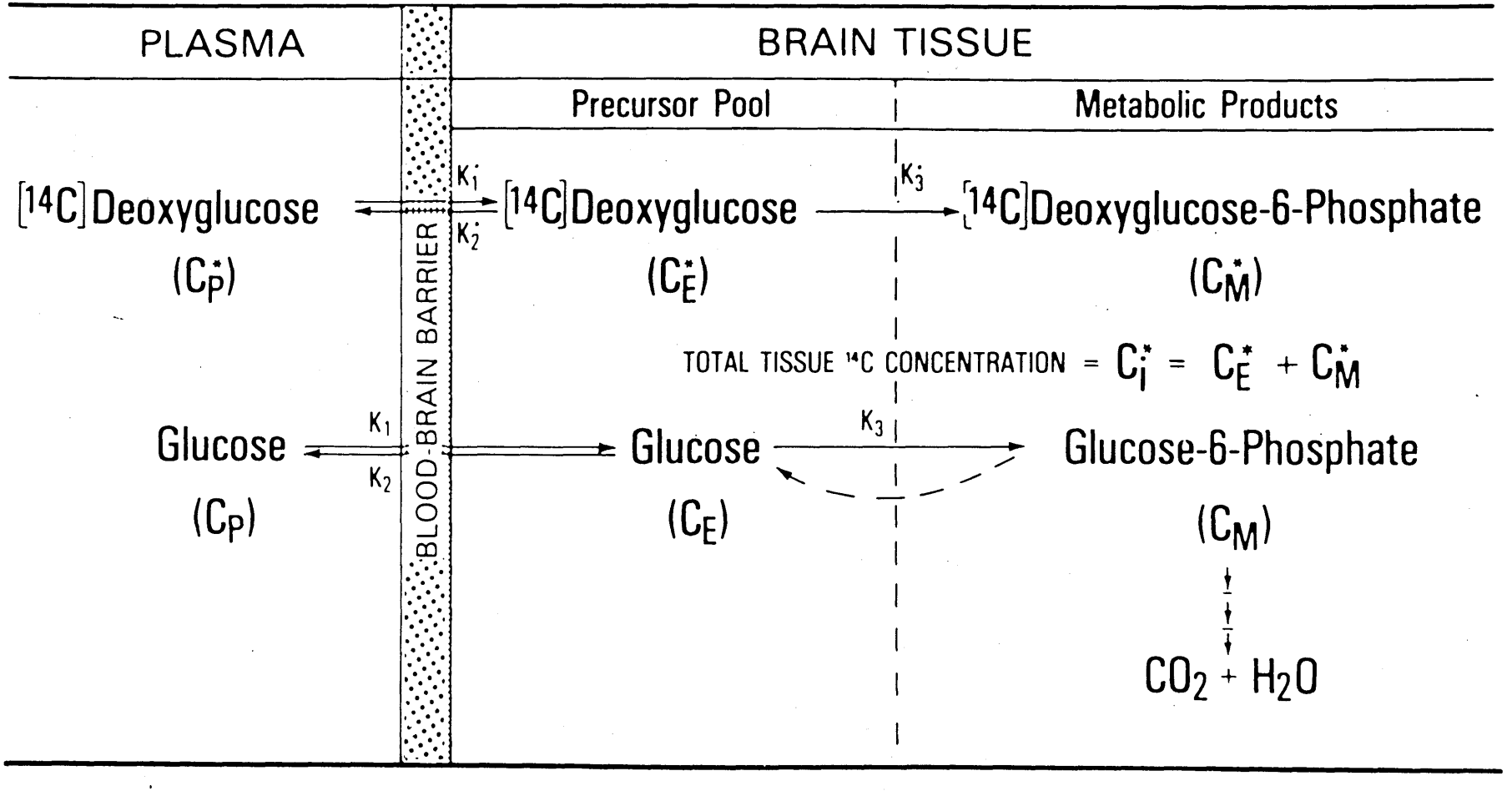
c) The ^{14}C-deoxyglucose and glucose concentrations in the arterial plasma are representative of those in cerebral capillaries.

The operational equation which allows the rate of glucose utilisation to be described mathematically is illustrated in Figure 15. Although the operational equation seems to be complex, it should be recognised that it is simply a specific form of a generally used equation by which the rates of the biochemical reaction with the tracer can be measured. The operational equation (Figure 15) can be written in a simple way as:-

$$\text{Rate of glucose utilisation} = \frac{\text{Total tissue } ^{14}\text{C} - \text{tissue 2-DG}}{\text{Lumped constant x integrated plasma specific activity.}}$$

Total ^{14}C in cerebral tissue is obtained via the densitometer; the tissue 2-deoxyglucose and integrated plasma activity are calculated from plasma histories of 2-deoxyglucose and glucose. The values for the rate constants and lumped constants for the operational equation were:-

Figure 14.


PLASMA
BRAIN TISSUE
Precursor Pool
Metabolic Products
[14C]Deoxyglucose
(C*P)
K1
K2
[14C]Deoxyglucose
(C*E)
K3
[14C]Deoxyglucose-6-Phosphate
(C*M)
TOTAL TISSUE 14C CONCENTRATION = C*i = C*E + C*M
BLOOD-BRAIN BARRIER
Glucose
(CP)
K1
K2
Glucose
(CE)
K3
Glucose-6-Phosphate
(CM)
CO2 + H2O

Legend to Figure 14.

Diagrammatic representation of the theoretical model. C_i^* represents the total ^{14}C concentration in a single homogeneous tissue of the brain. C_p^* and C_p represent the concentrations of $[^{14}C]$ deoxyglucose and glucose in the arterial plasma, respectively; C_E^* and C_E represent their respective concentrations in the tissue pools that serve as substrates for hexokinase. C_M^* represents the concentration of ^{14}C deoxyglucose 6-phosphate in the tissue. The constants k_1^*, k_2^*, and k_3^* represent the rate constants for carrier-mediated transport of $[^{14}C]$deoxyglucose from plasma to tissue, for carrier-mediated transport back from tissue to plasma, and for phosphorylation by hexokinase, respectively. The constants k_1, k_2 and k_3 are the equivalent constants for glucose. $[^{14}C]$Deoxyglucose and glucose share and compete for the carrier that transports both between plasma and tissue for hexokinase which phosphorylates them to their respective hexose 6-phosphates. The dashed arrow represents the possibility of glucose 6-phosphate hydrolysis by glucose 6-phosphatase activity, if any. (From Sokoloff et al 1977).

Figure 15.

General Equation for Measurement of Reaction Rates with Tracers:

$$\text{Rate of Reaction} = \frac{\text{Labeled Product Formed in Interval of Time, 0 to T}}{\left[\begin{array}{c}\text{Isotope Effect}\\ \text{Correction Factor}\end{array}\right]\left[\begin{array}{c}\text{Integrated Specific Activity}\\ \text{of Precursor}\end{array}\right]}$$

Operational Equation of [^{14}C]Deoxyglucose Method:

$$R_i = \frac{\overbrace{C_i^*(T)}^{\text{Total } {}^{14}C \text{ in Tissue at Time, T}} - \overbrace{k_1^* e^{-(k_2^*+k_3^*)T}\int_0^T C_p^* e^{(k_2^*+k_3^*)t}\,dt}^{{}^{14}C \text{ in Precursor Remaining in Tissue at Time, T}}}{\underbrace{\left[\frac{\lambda \cdot V_m^* \cdot K_m}{\phi \cdot V_m \cdot K_m^*}\right]}_{\text{Isotope Effect Correction Factor}}\underbrace{\left[\int_0^T \left(\frac{C_p^*}{C_p}\right)dt - e^{-(k_2^*+k_3^*)T}\int_0^T \left(\frac{C_p^*}{C_p}\right)e^{(k_2^*+k_3^*)t}\,dt\right]}_{\text{Correction for Lag in Tissue Equilibration with Plasma}}}$$

$\overbrace{\qquad\qquad\qquad\qquad}^{\text{Integrated Plasma Specific Activity}}$

Integrated Precursor Specific Activity in Tissue

Operational equation of radioactive deoxyglucose method and its functional anatomy. T represents the time at the termination of the experimental period; λ equals the ration of the distribution space of deoxyglucose in the tissue to that of glucose; ϕ equals the fraction of glucose which, once phosphorylated, continues down the glycolytic pathway; and K_m^*, V_m^* and K_m, V_m represent the familiar Michaelis-Menten kinetic constants of hexokinase for deoxyglucose and glucose, respectively. The other symbols are the same as those defined in Figure 14. (From Sokoloff, 1978).

	Gray Matter	White Matter
K_1^*	0.189 (min^{-1})	0.079 (min^{-1})
K_2^*	0.245 (min^{-1})	0.133 (min^{-1})
K_3^*	0.052 (min^{-1})	0.02 (min^{-1})

"Lumped Constant" - 0.483 for the albino rat,

(Sokoloff et al. 1977).

4.2 Practice.

Measurement of local cerebral glucose utilisation was performed on conscious rats, prepared as described previously, employing the ^{14}C-2-deoxyglucose technique (Sokoloff et al. 1977). For each experiment the isotope (50 μCi per animal), dissolved in saline, was given at a constant rate over 30 seconds. A total of 14 timed samples of arterial blood were withdrawn from the femoral cannulae and collected into heparinised plastic centrifuge tubes over the subsequent 45 minutes according to a pre-determined time schedule. The volume of the arterial samples was approximately 75 μl of whole blood, and this same volume was replaced by saline solution. The arterial blood plasma was separated by centrifugation within a few minutes of withdrawal. Of each plasma sample, 20 μl was pipetted from the centrifuge tubes into 1 ml of distilled water in 14 plastic scintillation vials. The glucose level was determined, using a 10 ul aliquot of plasma and a semi-automated glucose analyser (Beckman) with a glucose oxidase method. At the end of 45 minutes, the rat was killed by decapitation and the brain processed for quantitative autoradiography in the same manner as for the measurement of local

cerebral blood flow. The potential errors associated with the measurement and modification of the $[^{14}C]$-2-deoxyglucose technique have been discussed in detail elsewhere (Kelly and McCulloch, 1983b). Therefore, these errors were not studied in this thesis.

5. Neuropathological Studies

5.1 Perfusion fixation.

Perfusion fixation was performed in those animals used for histopathological assessment 3-4 hours after MCA occlusion. Approximately 3-5 min prior to sacrifice, anaesthesia was deepened by increasing the halothane concentration to 2%. A thoracotomy was made and the pericardium opened. The apex of the left ventricle was incised and a cannula was introduced into the ascending aorta. The tip of the right atrium was cut and body perfusion was performed with 40 ml of heparinised saline (1.5 ml heparin in 40 ml physiological saline). Fixation was then carried out with FAM (40% formaldehyde, glacial acetic acid and absolute methanol, 1:1:8 by volume). The FAM solution was infused slowly so that the arterial blood pressure was maintained within the range of the pre-sacrifice perfusion pressure and the rate adjusted by reference to the MABP trace. After infusion of 150-180 ml of FAM, the rat was decapitated and the head was stored in FAM for at least 12 hours. The brain was then removed and embedded in 10% formal saline for a minimum period of 24 hours at 40°C to ensure complete fixation.

5.2 Sectioning and staining for quantitative analysis.

The sectioning and quantification of ischaemic damage was performed according to the method of Graham et al. (1984). The animal was perfusion fixed with FAM. After removing the brain, the cerebral hemisphere was cut into four coronal slices of approximately equal thickness. These coronal slices were embedded in a paraffin wax block. Sections of 10 u thickness were taken at 250 u intervals through the block for histopathological examination. These sections were stained by a method combining cresyl violet and luxol fast blue, and haematoxylin and eosin. This method would produce multiple levels, each containing four different coronal sections. By this method the forebrain was effectively represented by about 36-48 equidistant coronal slices, thus enabling the extent of cellular ischaemic damage produced by middle cerebral artery occlusion to be assessed more fully than previously (Tamura et al. 1981a).

The histopathological changes were examined by conventional light microscopy. The brain sections showing ischaemic lesions were determined according to the histopathological criteria of Brown and Brierley (1968) in FAM perfusion-fixed animals. A good perfusion fixation was judged by the absence of intravascular blood, and the lack of cytological artefacts such as "dark cell" or "hydropic cells" (Cammermeyer, 1961; Brown and Brierley, 1968).

5.3 Determination of the area of ischaemic damage.

Areas of brain showing ischaemic cell damage were drawn directly on to eight stereotactically determined coronal levels

of the brain from an atlas of rat neuroanatomy (Konig and Klippel, 1963). The selected coronal levels with their anatomical location and anterior co-ordinates (mm) were as follows:-

1.	Intermediate olfactory tract	10.50
2.	Nucleus accumbens	8.92
3.	Septal nuclei	7.19
4.	Globus pallidus	6.06
5.	Anterior hypothalamus	5.15
6.	Lateral habenula	3.57
7.	Medial geniculate body	2.18
8.	Cerebral aqueduct	1.02

The areas of ischaemic damage which were drawn on to the map (Figure 16) were obtained by the image analyser function of the densitometer (Quantimet 720, Cambridge Instruments). The map was placed on an epidiascope stage and the image of the coronal sections was projected on to the densitometer screen. The area of the ischaemic cell damage was delineated by an electron "light" pen. The size of the area depicted was proportional to a picture point number displayed on the screen. A magnification of 360 picture points per mm^2 on the predetermined map was used.

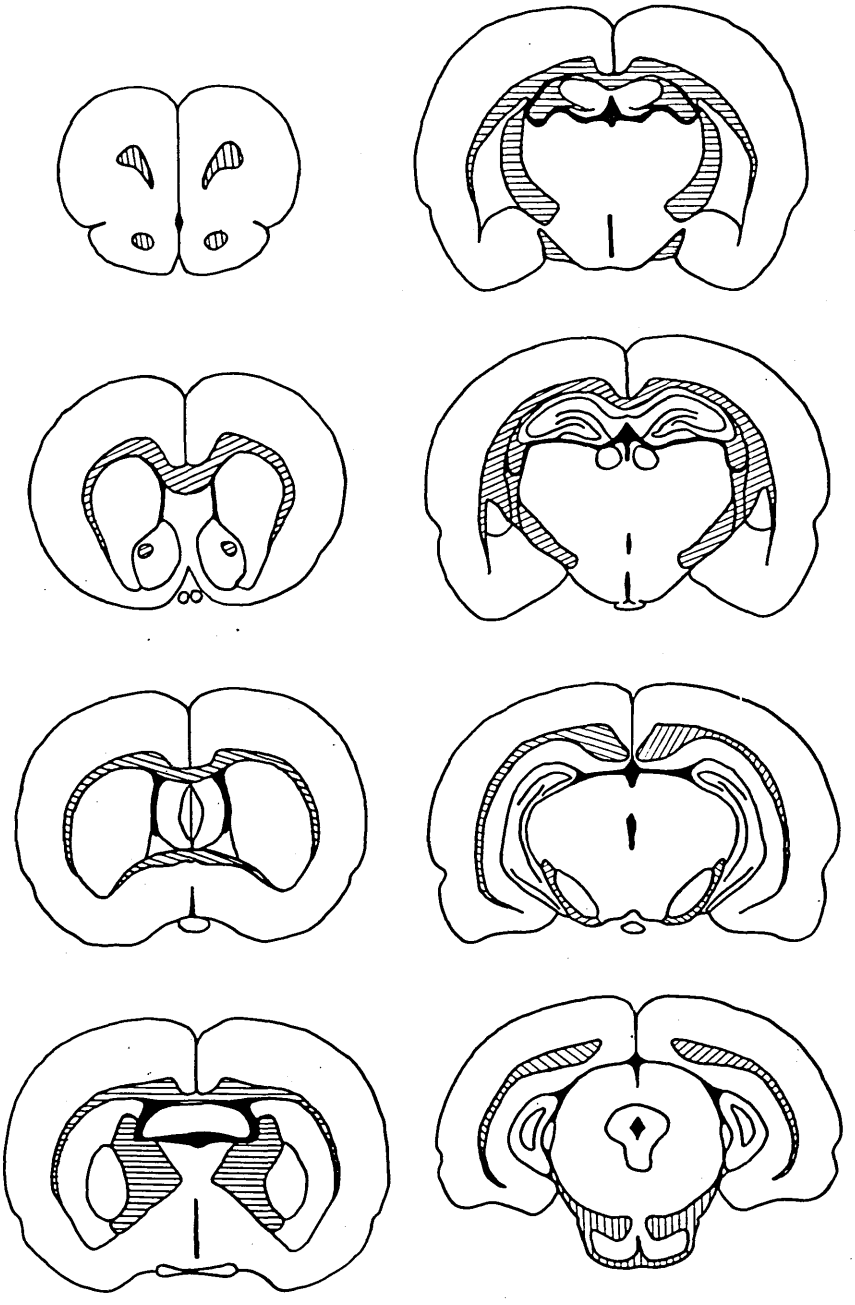
5.4 Measurement of the volume of ischaemic damage.

The total volume of the ischaemic damage was obtained by knowing:

a) the linear magnification of actual brain size (4X),

b) the magnification factor from map to densitometer (360 picture points per mm^2).

Figure 16.



Diagrammatic representation of coronal sections of rat brain which have been used for quantitative assessment of neuropathological damage following MCA occlusion.

c) The picture points numbers displayed on the screen which is proportional to the area of ischaemic cell damage.

The total volume was then calculated by plotting the areas of damage (mm^2) against the eight coronal sections with reference to distance between the sections (mm). A computer (Cromenco System 3) was used for deriving volumes of the ischaemic damage.

6. Experimental Design

6.1 The effect of vehicle and saline on physiological variables in anaesthetised rats.

The effect on the physiological variables of continuous infusion of the nimodipine solvent (lutrol-glycerine-water solvent) was examined. Following completion of the surgical preparation (see Chapter II, Section 1.1) at least 30 minutes were allowed to elapse before the infusion of the vehicle was started (n=6). Normal saline was infused into another series of animals (n=6). The volume of infusion was 0.0136 (ml kg^{-1} min^{-1}) for 60 minutes. Arterial blood samples were taken immediately before infusion of vehicle or saline, and in these were measured the PCO_2, PO_2, pH and plasma glucose concentration. thereafter the measurements were repeated at intervals of 30 minutes.

6.2 The effect of nimodipine on local CBF in anaesthetised rats.

The effect of continuous infusion of nimodipine (1, 2 or 4 μg $kg^{-1}min^{-1}$) on CBF was studied in anaesthetised, mechanically ventilated rats. Following completion of the surgical

preparation (see Section 1.1), at least 30 minutes were allowed to elapse before the start of infusion of the drug or vehicle. This was done to achieve normoxia, normocapnia, stability of the blood pressure, and normal plasma glucose concentration. Normocapnia was achieved by adjusting the tidal volume of the respirator. In control experiments (7 animals), the lutrol-glycerine-water solvent alone was infused via the femoral vein one hour before measuring local CBF. In nimodipine treated animals the rates of drug administration were 1 $\mu g\ kg^{-1}min^{-1}$ (6 animals), 2 $\mu g\ kg^{-1}min^{-1}$ (7 animals) and 4 $\mu g\ kg^{-1}min^{-1}$ (7 animals). CBF was measured 60 min after the start of infusion of the drug; the volume of infusion was 0.0136 ml $kg^{-1}min^{-1}$.

6.3 The effect of nimodipine on local CBF and local CGU relationship in conscious rats.

The effect of a continuous infusion of nimodipine on local CBF and local CGU was studied in conscious rats. In control experiments, rats were infused with the lutrol-glycerine-water vehicle and comparisons were made between these and rats treated with nimodipine (5 animals for CBF and 7 animals for CGU). The dose of nimodipine in each of these experiments was 1 $\mu g\ kg^{-1}\ min^{-1}$. The measurement periods of the two techniques differ (30 seconds for blood flow, 45 minutes for 2-deoxyglucose experiments), and the initiation of the experiments was therefore timed so that the median points of the measurements coincided. Thus, the experiments to determine local CBF were initiated 25 minutes after commencing the i.v. infusion of either nimodipine or vehicle, and those in which LCGU was measured were initiated 15 minutes

after commencing the infusion of either the nimodipine or the vehicle. Administration of the drug or solvent was continued until sacrifice of the animal. Although this experimental design aims to ensure that the measurements of blood flow and glucose use have as close a temporal similarity as possible, there are a number of attendant difficulties, in particular related to the drug infusion. For example, there is a difference of approximately 30% in the calculated arterial nimodipine concentration at the initiation of the CBF measurement compared to the value calculated at initiation of the measurement of glucose use (see Chapter I, Section 3.2.4). It should be recognised, however, that although the vasomotor effects of nimodipine in vitro are dose-related (Towart et al. 1982; Andersson et al. 1983), the relationship between the concentration of nimodipine administered and the alteration in cerebral blood flow in the intact animal is more complex because of the associated systemic hypotension induced by the drug (Haws et al. 1983; Mohamed et al. 1984; McCalden et al. 1984). These difficulties encountered in pharmacological investigations using the autoradiographic ^{14}C-2-deoxyglucose and ^{14}C-iodoantipyrine techniques are an inevitable consequence of the different periods over which the measurements are made, and are well recognised. The timing of the isotope administration in the present experimental design does provide the most representative determination of CBF for comparison with glucose utilisation, even although the latter is averaged over a longer period.

6.4 The effect of nimodipine on focal cerebral ischaemia.

6.4.1 The effect of pre-treatment with nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) on local CBF in anaesthetised rats following MCA occlusion.

The effect of pre-treatment with nimodipine (1 $\mu g\ kg^{-1}\ min^{-1}$) on local CBF in rats with MCA occlusion was examined. In control animals (5 animals) and in nimodipine pre-treated animals (5 animals), the vehicle and nimodipine were both infused for 30 minutes before MCA occlusion, and the infusion was continued for 30 minutes after the MCA occlusion. The nimodipine plasma concentration 60 minutes after infusion was calculated to be approximately 58 nM (see Chapter I, Section 3). The local CBF was measured as desribed (Chapter II, Section 2). In addition, the frequency distribution analysis of the local CBF was determined as described in Chapter II, Section 2.3.2.

6.4.2 The effect of pre-treatment with nimodipine (2 $\mu g\ kg^{-1}min^{-1}$) on local CBF in anaesthetised rats.

The effect on local CBF of pre-treatment with continuous intravenous nimodipine (2 $\mu g\ kg^{-1}min^{-1}$) was examined in rats with middle cerebral artery occlusion. In control untreated animals (9 animals) and in nimodipine treated animals (7 animals) the infusion was commenced 30 minutes before MCA occlusion and continued for 30 minutes thereafter.

6.4.3 The effect of pre-treatment with nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) on the neuropathological findings in anaesthetised rats following MCA occlusion.

The effect of pre-treatment with nimodipine (1 $\mu g\ kg^{-1}\ min^{-1}$) on the histological findings in rats after MCA occlusion was investigated. In control (n=8) and nimodipine treated animals (n=8), the infusion of the drug or vehicle was started 30 minutes before MCA occlusion and continued for at least 3-4 hours, at which time perfusion fixation with FAM was performed. Measurements of brain sections and the volumes of brain showing ischaemic cell damage were made by a quantitative method (see Chapter II, Section 5).

The assessment of histopathological changes was conducted without prior knowledge of the schedule of treatment. Each experiment was given a code number. After obtaining the results of each experiment, the code for the whole series was broken and the statistical analysis of the results was performed thereafter.

6.4.4 Effect of post-treatment with nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) on local CBF in rats following MCA occlusion.

The measurement of local CBF was performed 35 minutes after occlusion of MCA. In five experimental animals, intravenous infusion of nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) was started 5 minutes after MCA occlusion and was continued for 30 minutes until sacrifice. In the 5 control animals a similar volume of the solvent was used. Arterial blood samples were taken immediately before occlusion of the artery, and immediately before measurement of CBF for the determination of PaO_2, $PaCO_2$, pH and plasma glucose concentration.

7. Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). In the blood flow investigations, unpaired Student's t-test was used for comparison between the vehicle infused and nimodipine treated animals. For comparison between ipsilateral and contralateral side to the middle cerebral artery occlusion, paired Student's t-test was used. The multiple, non-independent measurements of the brain regions in each animal and various dosages of the drug administered in this study need more conservative statistical treatment. The Bonferroni correction factor, which has been suggested to reduce the error which may occur in using multiple t-testing (Wallenstein et al. 1980; Ford, 1983), has been used.

The analysis of the relationship between cerebral blood flow and glucose utilisation, the gradient (m) and coefficient of correlation (r) were derived from linear regression analysis to obtain the best fit of the data to the line ($y=mx+b$). Although this form of data analysis is not appropriate for rigorous statistical analysis, it does provide an easily assimilated graphic display (see McCulloch et al. 1982). Therefore, the relationship between the natural logarithms of mean LCBF and LCGU in control animals and animals that received nimodipine was used.

In the neuropathological studies, the a priori hypothesis that nimodipine reduced the volume of ischaemic damage in the cerebral cortex was tested with one-tailed Student's t-test. The possibility that the area of ischaemic damage at each stereotactic level was altered by nimodipine pretreatment was examined with the more conservative two-tailed Student's t-test.

CHAPTER III

RESULTS

1. Effect of Vehicle and Saline Administration on Physiological Variables in Anaesthetised Rats

The majority of animals used as a control in this thesis were receiving vehicle (lutrol-glycerine-water solvent). Some animals used as a control in ischaemia received saline. In these animals the effect of pre-treatment of continuous intravenous infusion of nimodipine (2 $\mu g\ kg^{-1} min^{-1}$: see Chapter III, Section 3.2) on local CBF was investigated. Therefore, twelve animals (weighing between 370 and 480g) were used to find whether the administration of vehicle or saline produced any changes in the MABP, blood gas tensions, plasma glucose concentrations and arterial pH. There were no significant alterations in PCO_2, PO_2, arterial plasma glucose concentrations and arterial pH in any groups (Table 15). The infusion of either lutrol-glycerine-water solvent or physiological saline did not significantly change the MABP (Table 16).

TABLE 15. ARTERIAL BLOOD VARIABLES

Variable	Vehicle (n=6)			Saline (n=6)		
	Base Line	at 30 min	at 60 min	Base Line	at 30 min	at 60 min
PCO_2 (mm Hg)	37 ± 1	35 ± 1	36 ± 1	37 ± 1	36 ± 2	36 ± 3
PO_2 (mm Hg)	125 ± 6	130 ± 5	134 ± 8	125 ± 9	133 ± 8	128 ± 7
Arterial plasma glucose concentration (mM)	9.0 ± 0.02	8.5 ± 0.02	8.9 ± 0.1	9 ± 0.03	8.8 ± 0.02	9.0 ± 0.04
pH	7.439 ± 0.01	7.424 ± 0.01	7.440 ± 0.01	7.434 ± 0.01	7.438 ± 0.01	7.425 ± 0.01

Data presented were measured before starting the infusion of vehicle or saline, and at 30 min and 60 min during the course of the experiment. Values are mean ± SEM.

TABLE 16. THE TIME COURSE OF THE EFFECTS OF VEHICLE AND SALINE INFUSION ON MABP.

	MABP Base Line	MABP at 5 min	MAPB at 15 min	MABP at 30 min	MABP at 45 min	MABP at 60 min
Vehicle n=6	108 ± 4	107 ± 3	100 ± 6	107 ± 3	105 ± 3	105 ± 3
Saline	100 ± 8	102 ± 4	103 ± 6	101 ± 4	102 ± 3	103 ± 3

Data are presented as mean ± SEM, MABP (mm Hg)

2. The Effects of Nimodipine on the Cerebral Circulation in Normal Brain

2.1 Effect of nimodipine on local CBF in anaesthetised rats: relationship to arterial blood pressure.

2.1.1 General: There were no significant changes in arterial PCO_2, PO_2 or pH in any of the groups during infusion of the drug (Table 17). The PCO_2 in all groups varied between 34 ± 1 to 36 ± 1; PO_2 between 124 ± 6 to 134 ± 4, and the pH between 7.419 ± 0.01 to 7.440 ± 0.001. The infusion of a lutrol-glycerine-water solvent in control animals did not significantly change either mean arterial blood pressure or arterial plasma glucose.

Blood pressure: By contrast, nimodipine administration produced a dose-dependent decrease in MABP, with the maximum decrease being observed at five minutes from the start of the infusion (Table 18, Figure 17). Thereafter the MABP rose, but stabilised at a level below the control value by an extent proportional to the dose that was used. For example, after 15 min infusion with the lowest concentration of nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) blood pressure was reduced only modestly (about 14%), and a similar decrease (16%) was seen at a dose of 2 $\mu g\ kg^{-1}\ min^{-1}$. However, a dose of 4 $\mu g\ kg^{-1}min^{-1}$ reduced blood pressure markedly (by 26%).

A dose-dependent increase in plasma glucose was also observed during the infusion of nimodipine (Figure 17). An insignificant elevation in plasma glucose was obtained during (1 $\mu g\ kg^{-1}min^{-1}$) infusion, but significant increases occurred with 2 $\mu g\ kg^{-1}min^{-1}$ (by 23%) and with 4 $\mu g\ kg^{-1}min^{-1}$ of nimodipine (by 44%).

2.1.2 The effect on local CBF.

The intravenous infusion of nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) provoked significant increases in local CBF in 9 of the 31 regions (including the cerebral cortex, hippocampus, hypothalamus and most thalamic nuclei). These changes reached significant levels ($p < 0.05$ or $p < 0.01$) with Student's t-test for grouped data with Bonferroni correction (Table 19). Trends towards an increase in CBF, which failed to reach significance with this conservative statistical analysis, but which would have attained significance ($p < 0.05$) with uncorrected t-test, were noted in four brain regions (thalamus mediodorsal, septal nucleus, caudate nucleus and globus pallidus; Table 19).

Administration of nimodipine (2 $\mu g\ kg^{-1}min^{-1}$) increased significantly the local CBF in parietal cortex, sensory-motor cortex, auditory cortex and frontal cortex, and to a lesser extent in nucleus accumbens and medial geniculate.

With four-fold increase in the concentration of intravenous nimodipine administration (4 $\mu g\ kg^{-1}min^{-1}$) the local CBF was increased significantly in parietal cortex, sensory-motor cortex, auditory cortex and frontal cortex. Trends of increase in local CBF in medial geniculate, hippocampus and septal nucleus were again observed.

It was noteworthy that the increases in CBF were most marked and the significance levels greatest during infusion of the lowest studied concentration of nimodipine (Table 19). There was not a dose-dependency (i.e., with further increases in CBF being observed during infusion of greater concentrations of nimodipine) in the range studied in any region of the brain. For example, the CBF of the parietal cortex increased by 108%, 91% and 80% with 1, 2 and 4 $\mu g\ kg^{-1}min^{-1}$ of nimodipine infusion,

respectively.

The effects of nimodipine in local CBF were regionally heterogeneous. The most marked increases in CBF (by more than 60%) were observed in rostral neocortex; moderate increases (25-50%) were observed in various subcortical forebrain areas (caudate nucleus, globus pallidus, hypothalamus and some thalamic nuclei) and some components of the limbic system (hippocampus, septal nucleu and amygdala). In contrast to the increases in CBF observed in forebrain regions, there was no significant increase in regions of the lower brain stem, cerebellum and pons or in myelinated fibre tracts (Table 19).

TABLE 17. PHYSIOLOGICAL VARIABLES

	Vehicle (n=7)	Nimodipine		
		1 $\mu g\ kg^{-1}min^{-1}$ (n=6)	2 $\mu g\ kg^{-1}min^{-1}$ (n=7)	4 $\mu g\ kg^{-1}min^{-1}$ (n=7)
PCO_2	34 ± 1	34 ± 1	36 ± 1	36 ± 1
PO_2	134 ± 4	124 ± 6	134 ± 8	128 ± 7
pH	7.440 ± 0.001	7.439 ± 0.01	7.432 ± 0.01	7.419 ± 0.01
Arterial plasma glucose	8.9 ± 0.1	9.0 ± 0.4	11 ± 0.02*	13 ± 0.03*

Data presented were measured 5 min prior to the determination of CBF: Mean ± SE.

*$p < 0.05$. Student's t-test with Bonferroni correction.

TABLE 18. TIME COURSE OF THE EFFECT OF NIMODIPINE ON MABP.

Nimodipine concentration	Base line MABP	MABP at 5 mins	MABP at 15 mins	MABP at 30 mins	MABP at 45 mins	MABP at 60 mins
1 $\mu g\ kg^{-1}min^{-1}$	108 ± 4	81 ± 5*	93 ± 4*	93 ± 4*	93 ± 4*	93 ± 4*
2 $\mu g\ kg^{-1}min^{-1}$	100 ± 6	81 ± 4*	84 ± 4*	84 ± 4*	84 ± 4*	84 ± 4*
4 $\mu g\ kg^{-1}min^{-1}$	101 ± 5	72 ± 4*	73 ± 4*	74 ± 4*	75 ± 4*	75 ± 4*

Data are presented as mean ± SE (mm Hg). *$p < 0.05$ from base line.

Student's paired t-test with Bonferroni correction.

TABLE 19. EFFECTS OF NIMODIPINE ON LOCAL CBF.

Structure	Vehicle (n=7)	Nimodipine		
		1 μg $kg^{-1}min^{-1}$ (n=6)	2 μg $kg^{-1}min^{-1}$ (n=7)	4 μg $kg^{-1}min^{-1}$ (n=7)
Parietal Cortex	133 ± 7	276 ± 20**	254 ± 21**	239 ± 23**
Sensory-motor Cortex	109 ± 15	253 ± 14**	274 ± 32**	253 ± 20**
Auditory Cortex	162 ± 17	289 ± 21**	247 ± 21*	256 ± 21*
Frontal Cortex	147 ± 8	242 ± 22*	217 ± 22*	244 ± 19*
Nucleus Accumbens	132 ± 9	218 ± 11**	179 ± 19#	184 ± 24
Medial Geniculate	155 ± 9	254 ± 18**	217 ± 27#	205 ± 20#
Hypothalamus	94 ± 3	126 ± 7*	108 ± 10	109 ± 13
Hippocampus	122 ± 9	195 ± 18*	188 ± 30	171 ± 18#
Amygdala	99 ± 4	130 ± 9*	122 ± 14	119 ± 12
Thalamus (MD)	135 ± 18	187 ± 19#	170 ± 20	158 ± 12
Septal Nucleus	97 ± 5	132 ± 12#	119 ± 13	122 ± 8#
Caudate Nucleus	163 ± 14	209 ± 9#	187 ± 21	190 ± 15
Globus Pallidus	74 ± 3	92 ± 5#	85 ± 11	84 ± 8
Dentate Gyrus	124 ± 7	152 ± 12	153 ± 22	151 ± 5
Inferior Colliculus	212 ± 15	273 ± 28	298 ± 36	259 ± 16
Visual Cortex	162 ± 24	198 ± 28	210 ± 28	211 ± 24
Thalamus (VL)	115 ± 6	120 ± 9	131 ± 19	125 ± 9
Habenula	172 ± 11	228 ± 20	197 ± 21	206 ± 18

TABLE 19 (Contd.).

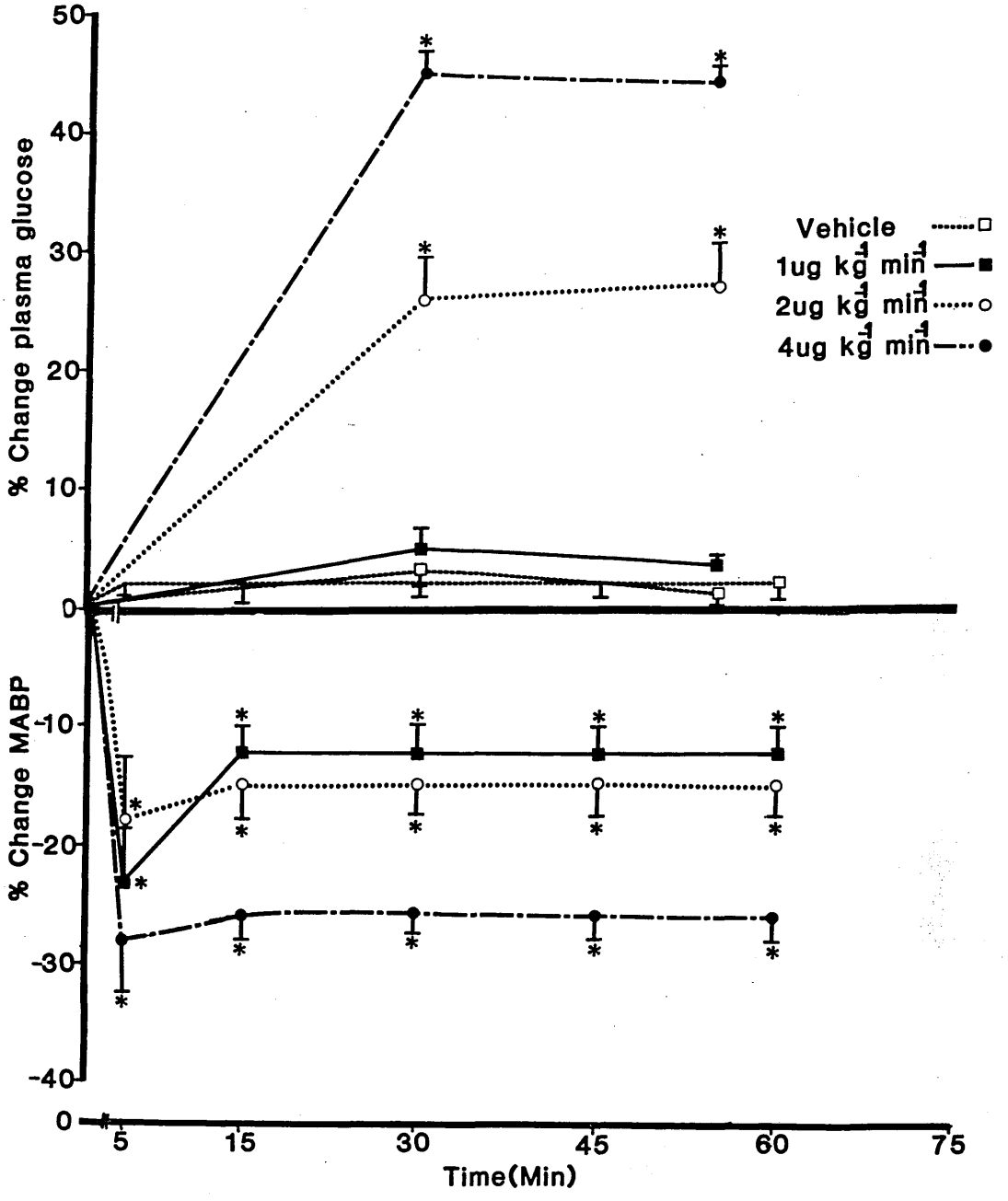
Structure	Vehicle (n=7)	Nimodipine		
		1 μg $kg^{-1}min^{-1}$ (n=6)	2 μg $kg^{-1}min^{-1}$ (n=7)	4 μg $kg^{-1}min^{-1}$ (n=7)
Red Nucleus	170 ± 10	202 ± 23	184 ± 23	173 ± 13
Substantia Nigra Compacta	109 ± 20	137 ± 7	123 ± 10	141 ± 11
Substantia Nigra Reticulata	104 ± 5	102 ± 5	90 ± 5	109 ± 12
Vestibular Nucleus	200 ± 17	193 ± 25	193 ± 22	220 ± 21
Cochlear Nucleus	207 ± 6	213 ± 12	229 ± 30	232 ± 15
Superior Olive	180 ± 12	198 ± 21	218 ± 28	202 ± 15
Superior Colliculus	158 ± 6	206 ± 20	185 ± 19	185 ± 14
Pons	114 ± 7	115 ± 8	138 ± 18	135 ± 9
Cerebellar Nucleus	206 ± 17	185 ± 19	212 ± 24	221 ± 17
Cerebellar White Matter	58 ± 7	55 ± 3	58 ± 6	68 ± 8
Cerebellar Hemisphere	103 ± 8	91 ± 7	102 ± 10	111 ± 14
Genu	53 ± 7	65 ± 6	73 ± 11	60 ± 4
Internal Capsule	50 ± 5	53 ± 5	50 ± 6	49 ± 2

Local CBF (ml $100g^{-1}min^{-1}$) is presented as mean ± SE and was measured after 60 minutes of infusion at the rates shown.

**$p<0.01$, *$p<0.05$. Student's t-test for grouped data with Bonferroni correction.

Increases in CBF which failed to achieve significance with this conservative procedure, but which would have attained significance ($p<0.05$) with uncorrected t-test, are indicated (#). The data are ranked as a hierarchy from regions in which the response was most significant (parietal cortex) to regions in which it was least significant (internal capsule).

Figure 17.



Time course of the effect of a continuous infusion of vehicle or nimodipine upon arterial blood pressure and plasma glucose. Data are presented as percent of pre-infusion base line value (mean $\pm$ SE). *$p < 0.05$ from base line (Student's paired t-test with Bonferroni correction).

2.2 Effect of nimodipine on local CBF and local CGU relationship in conscious rats.

2.2.1 General:

There were no significant changes in PCO_2, PO_2 or pH during the administration of the drug in any of the experimental groups (Table 20). In groups used for measurement of blood flow, the PCO_2 was 36 ± 2 and 35 ± 1 for control and nimodipine infusion, respectively; PO_2 was 81 ± 2 for vehicle and 82 ± 1 for nimodipine. The plasma glucose was 8.3 ± 0.06 mM (vehicle) and 8.5 ± 0.11 (nimodipine).

In groups used for the measurement of glucose utilisation (Table 20), PCO_2 was 35 ± 2 (vehicle) and 36 ± 2 (nimodipine), and PO_2 was 81 ± 1 (vehicle) and 83 ± 3 (nimodipine). Plasma glucose was measured as 8.4 ± 0.05 nM (vehicle) and 8.3 ± 0.03 mM (nimodipine).

The infusion of a lutrol-glycerine-water solvent in control animals did not change the MABP significantly, whereas with nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) there was a small reduction in MABP (8%) which just failed to achieve statistical significance (Table 20).

2.2.2 Results

The effects of nimodipine infusion on local CBF and local CGU.

Cerebral glucose utilisation: The local CGU was measured in 34 regions of the brain as shown in Table 21. Intravenous infusion of nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) did not alter significantly the rate of glucose utilisation in any of the 34 regions of the CNS which were examined (Table 21). Even though in many regions there was a tendency towards slightly lower (5-10%) rates of glucose use during nimodipine infusion, the responses were

similar in quantitative terms, and the autoradiograms from nimodipine-treated rats were indistinguishable from those animals which had received vehicle. Even after detailed visual inspection of the autoradiograms, no region could be identified as displaying a focal alteration in glucose utilisation.

Local cerebral blood flow: Cerebral blood flow was significantly increased during nimodipine (1 $\mu g\ kg^{-1} min^{-1}$) administration in 24 of 34 regions of the CNS examined (Table 21, Figures 18-20), most notably the cerebral cortices (auditory cortex, visual cortex, parietal cortex, frontal cortex and sensory-motor cortex), thalamus, hypothalamus, most extrapyramidal regions, and some components of the limbic system such as the hippocampus (molecular layer) and amygdala. In a few cerebral structures (e.g., globus pallidus, pontine reticular formation) the level of local CBF was minimally altered by nimodipine administration. The overall unweighted average of cerebral blood flow in gray matter areas was increased by 44% during the infusion of nimodipine.

The median alteration in local CBF provoked by nimodipine administration was 32%, with the proportionately greatest increases being observed in the posterior parietal cortex (increased by 84%), auditory cortex (by 64%), medial geniculate body (by 61%) and sensory-motor cortex (by 50%), and the proportionately smallest increases being observed in the globus pallidus (3%) and pontine reticular formation (12%). Blood flow in each of the four regions of the white matter was unaltered by nimodipine infusion (mean increase in CBF: 1%).

Relationship between local blood flow and local glucose utilisation: In vehicle-treated control rats there is an excellent correlation ($p<0.01$) between the local levels of blood flow and glucose utilisation (Figures 18 and 19). This was observed by analysis of data using linear regression; evaluation of the derived correlation coefficient revealed a close correlation between the mean levels of cerebral blood flow (Y) and the mean levels of glucose utilisation (X) in all regions of the CNS (Figure 18), the correlation coefficient to the least squares fit of $Y = mx+b$ being 0.95 ($p<0.01$), and m = 1.5 (ml blood/μmol glucose), with the highest levels of each variable being noted in cerebral cortex, thalamus and hippocampal formation, and the lowest values in the hypothalamus, amygdala and white matter (Table 21). The level of blood flow (ml $100g^{-1}min^{-1}$) was approximately 1.5 times the level of glucose use (μmol $100g^{-1}min^{-1}$) in all regions of vehicle-treated control animals. No significant inter-regional differences could be demonstrated in any region in the value of local flow: glucose ratio, using the conservative test of Greenhouse and Geisser (for details see McCulloch et al. 1982).

During nimodipine infusion there was a close correlation between local CBF and local CGU ($y=mx+b$), being 0.95 for both nimodipine and vehicle groups ($p<0.01$) (Figure 18), but the relationship between local CBF and local CGU was re-set. Thus, for a given level of LCGU there was a higher level of local cerebral blood flow.

The disturbance of the relationship by nimodipine could be easily seen from the median value of the ratio of local blood flow to glucose use (2.5 ml.μmol^{-1}). Although during treatment with nimodipine the local levels of blood flow and glucose use

generally displayed the same regional hierarchies, inter-regional differences which just achieved statistical significance ($p<0.05$) with the test of Greenhouse and Geisser could be demonstrated in the local ratio of blood flow to glucose use. These were restricted to two regions of gray matter, namely sensory-motor cortex (flow:glucose ratio 3.69 ml μmol^{-1} which was significantly greater than that in other areas with the same treatment), and globus pallidus (ratio 1.75 ml μmol^{-1}, significantly lower than in other areas with the same treatment). Notwithstanding this evidence for slight regional heterogeneity in the local flow - glucose use relationship with nimodipine treatment, it should be emphasised that flow and glucose use remained generally strongly correlated.

Because the above data were derived from two different sample populations, in which two distinct measurements were made, the relationship between local CBF and local CGU was re-examined using a rigorous statistical model developed by McCulloch et al. (1982) to investigate the relationship of the average local CGU in different regions, but taking into account the correlation between observations in the same animals. The transformation of the data to natural logarithms of the mean values disclosed a linear relationship between local CBF and local CGU. This is characterised by unit slope and intercept $\propto$ (Figure 20), and the coupling between local CBF and local CGU in nimodipine treatment is seen to be re-set towards increased values for cerebral blood flow at a very high level of significance ($p = 0.0002$).

TABLE 20. PHYSIOLOGICAL VARIABLES.

		MABP	PCO_2	PO_2	pH	Plasma Glucose
Groups used for measurement of blood flow	Vehicle, n=6	124 ± 6	36 ± 2	81 ± 2	7.43 ± 0.015	8.3 ± 0.06
	Nimodipine, n=7	114 ± 3	35 ± 1	82 ± 1	7.42 ± 0.005	8.5 ± 0.11
Groups used for measurement of glucose utilisation.	Vehicle, n=5	125 ± 5	35 ± 2	81 ± 1	7.44 ± 0.004	8.4 ± 0.05
	Nimodipine, n=5	114 ± 2	36 ± 2	83 ± 3	7.43 ± 0.010	8.3 ± 0.03

Cardiovascular and respiratory status of animals in which LCBF and LCGU were determined.
Data area means ± SE, and represent the levels measured immediately prior to the measurement.
There are no significant differences between the groups (unpaired Student's t-test).

TABLE 21. THE EFFECTS OF NIMODIPINE (1 μg $kg^{-1}min^{-1}$, i.v.) UPON LOCAL CEREBRAL GLUCOSE UTILISATION AND LOCAL CEREBRAL BLOOD FLOW.

	Cerebral Glucose Utilisation		Cerebral Blood Flow	
Region	Vehicle n:6	Nimodipine n:7	Vehicle n:5	Nimodipine n:5
Visual cortex	98 ± 5	92 ± 3	140 ± 15	186 ± 9[a]
Auditory cortex	127 ± 8	115 ± 4	188 ± 14	309 ± 22[b]
Parietal cortex	96 ± 5	89 ± 4	178 ± 12	329 ± 13[c]
Sensory-motor cortex	96 ± 4	94 ± 4	165 ± 16	248 ± 13[b]
Frontal cortex	91 ± 3	90 ± 2	179 ± 17	249 ± 12[b]
Thalamus (mediodorsal)	76 ± 3	70 ± 1	140 ± 11	169 ± 3[a]
Thalamus (ventrolateral)	63 ± 3	59 ± 2	124 ± 6	152 ± 6[a]
Habenula	87 ± 8	82 ± 5	161 ± 16	216 ± 8[a]
Medial geniculate body	94 ± 5	87 ± 3	160 ± 11	243 ± 20[b]
Lateral geniculate body	72 ± 5	66 ± 2	113 ± 6	182 ± 15[b]
Hypothalamus	47 ± 1	50 ± 2	92 ± 4	131 ± 5[c]
Hippocampus	77 ± 2	75 ± 2	118 ± 9	161 ± 12[a]
Dentate gyrus	66 ± 4	62 ± 1	105 ± 5	131 ± 15
Amygdala	48 ± 1	50 ± 3	95 ± 6	121 ± 3[b]
Septal nucleus	50 ± 2	50 ± 1	96 ± 11	125 ± 14
Caudate nucleus	91 ± 4	82 ± 2	136 ± 12	183 ± 16[a]
Nucleus accumbens	87 ± 3	82 ± 3	147 ± 15	202 ± 16[a]

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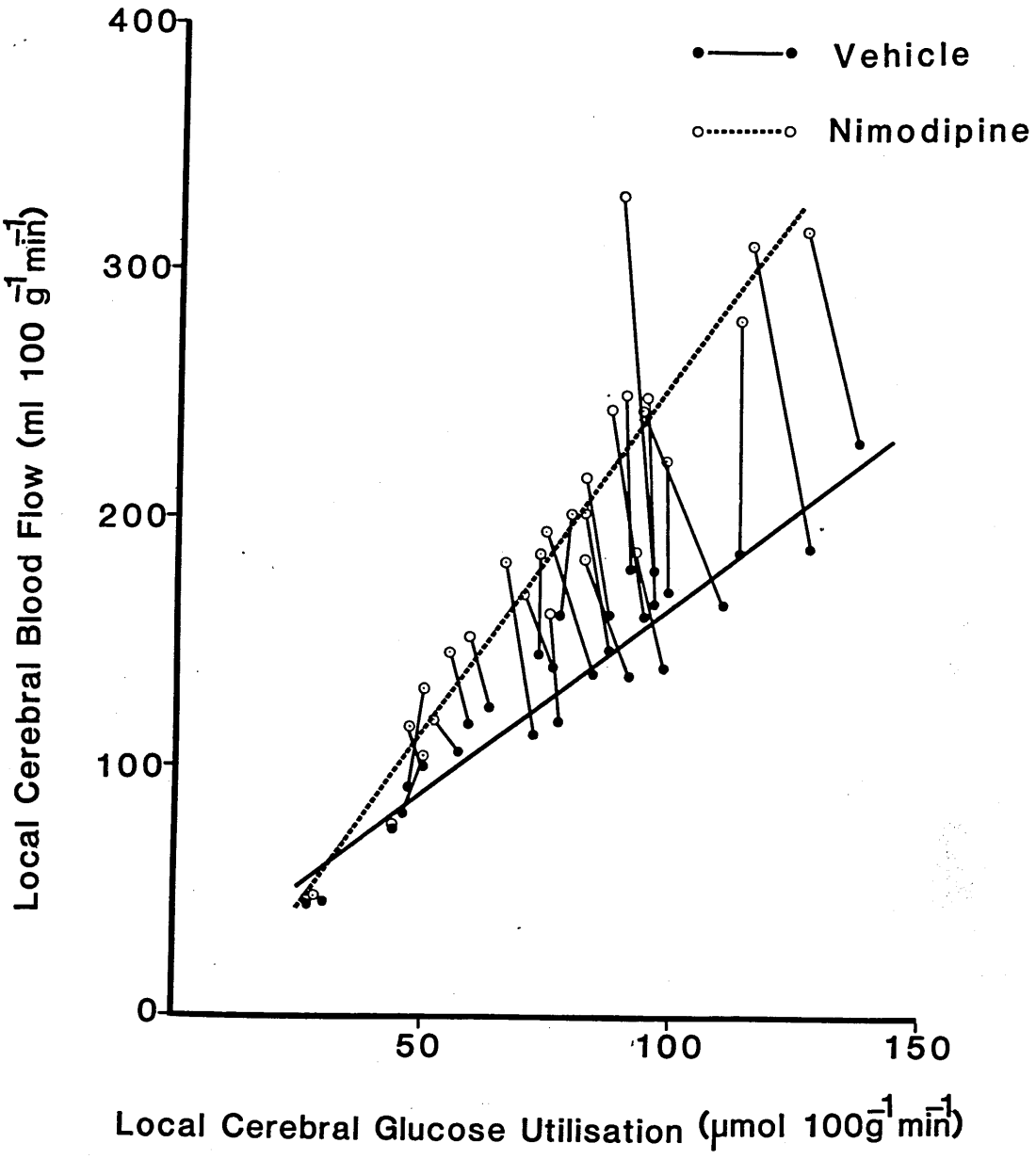
TABLE 21 (Contd.).

Region	Cerebral Glucose Utilisation		Cerebral Blood Flow	
	Vehicle n:6	Nimodipine n:7	Vehicle n:5	Nimodipine n:5
Globus pallidus	44 ± 1	44 ± 3	75 ± 5	77 ± 1
Red nucleus	73 ± 5	73 ± 3	145 ± 10	185 ± 5[b]
Substantia nigra (pars compacta)	59 ± 2	55 ± 1	117 ± 5	146 ± 4[b]
Substantia nigra (pars reticulata)	50 ± 2	47 ± 1	100 ± 2	116 ± 5[a]
Subthalamic nuclei	75 ± 3	68 ± 3	138 ± 11	175 ± 6[a]
Vestibular nucleus	99 ± 3	98 ± 5	170 ± 12	223 ± 13[a]
Cochlear nucleus	113 ± 5	113 ± 5	186 ± 11	279 ± 14[c]
Superior olive	110 ± 6	93 ± 5	165 ± 8	243 ± 19[b]
Inferior colliculus	137 ± 10	126 ± 8	230 ± 22	315 ± 19[a]
Superior colliculus	84 ± 5	74 ± 2	137 ± 7	194 ± 14[b]
Pons	57 ± 2	52 ± 2	106 ± 5	119 ± 6
Cerebellar hemisphere	46 ± 1	50 ± 3	81 ± 8	104 ± 5
Cerebellar nuclei	77 ± 4	79 ± 3	161 ± 14	201 ± 15
Cerebellar white	29 ± 1	27 ± 1	49 ± 1	46 ± 1
Corpus callosum	30 ± 1	27 ± 1	47 ± 3	48 ± 1
Genu of corpus callosum	30 ± 1	28 ± 1	46 ± 3	48 ± 1
Internal capsule	27 ± 1	27 ± 1	45 ± 1	47 ± 1

The effects of nimodipine (1 μg $kg^{-1}min^{-1}$) upon LCBF and LCGU. (a) $p<0.05$, (b) $p<0.01$, (c) $p<0.001$, for the comparison between vehicle and nimodipine administration (unpaired Student's t-test).
Data are presented as mean ± SE; glucose utilisation (μmol $100g^{-1}min^{-1}$); blood flow (ml $100g^{-1}min^{-1}$).

Figure 18.



The relationship between mean LCBF and mean LCGU in vehicle-treated control animals (left) and animals that received nimodipine (right). The gradient (m) and coefficient of correlation (r) derived from linear regression analysis are m = 1.5; r = 0.95 for vehicle, and m = 2.8; r = 0.95 for nimodipine.

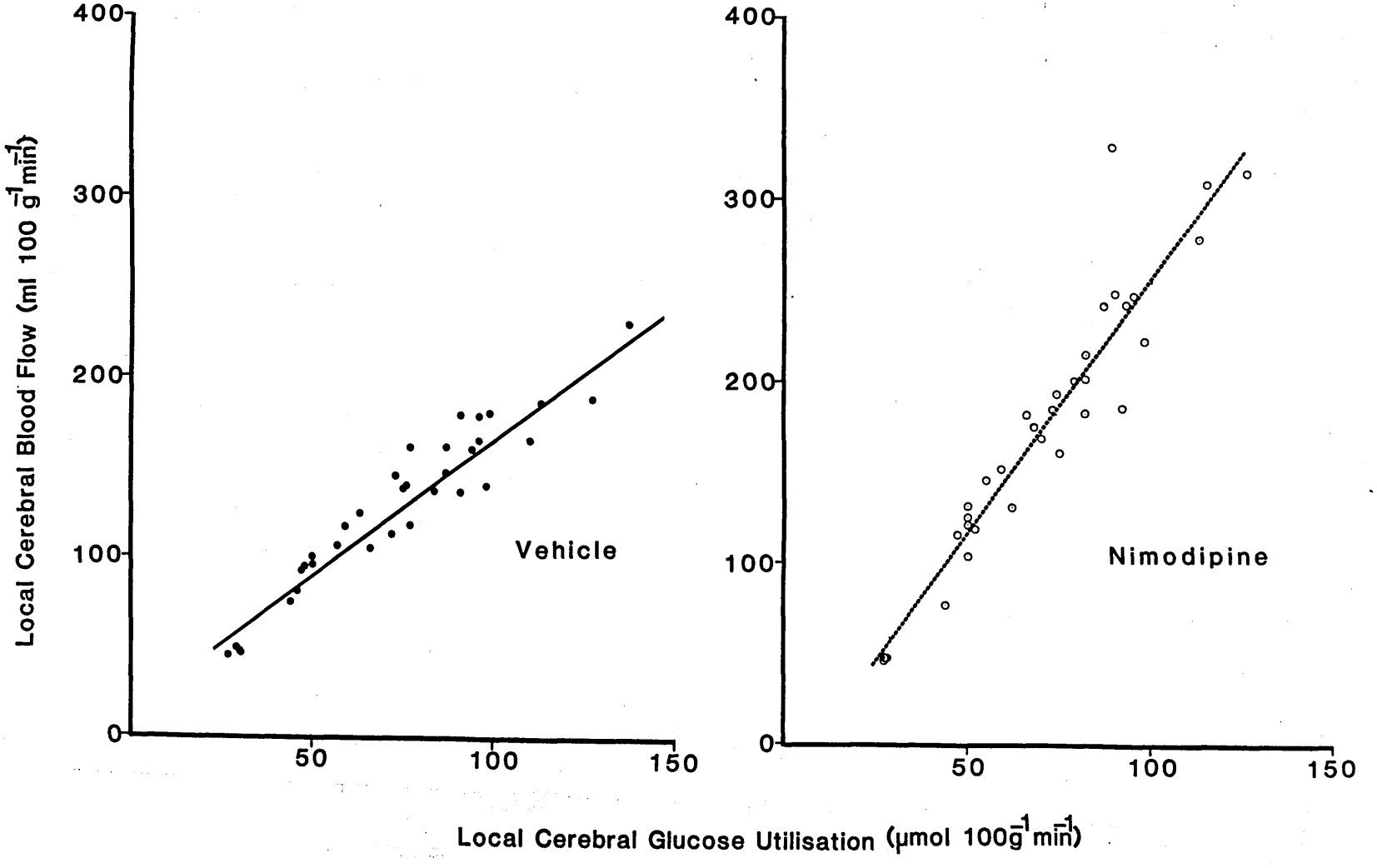


Figure 19. Relationship between LCBF and LCGU in animals which received vehicle (•) and those which received nimodipine (o). The gradient (m) and coefficient of correlation (r) derived from linear regression are indicated in the legend to Figure 18.

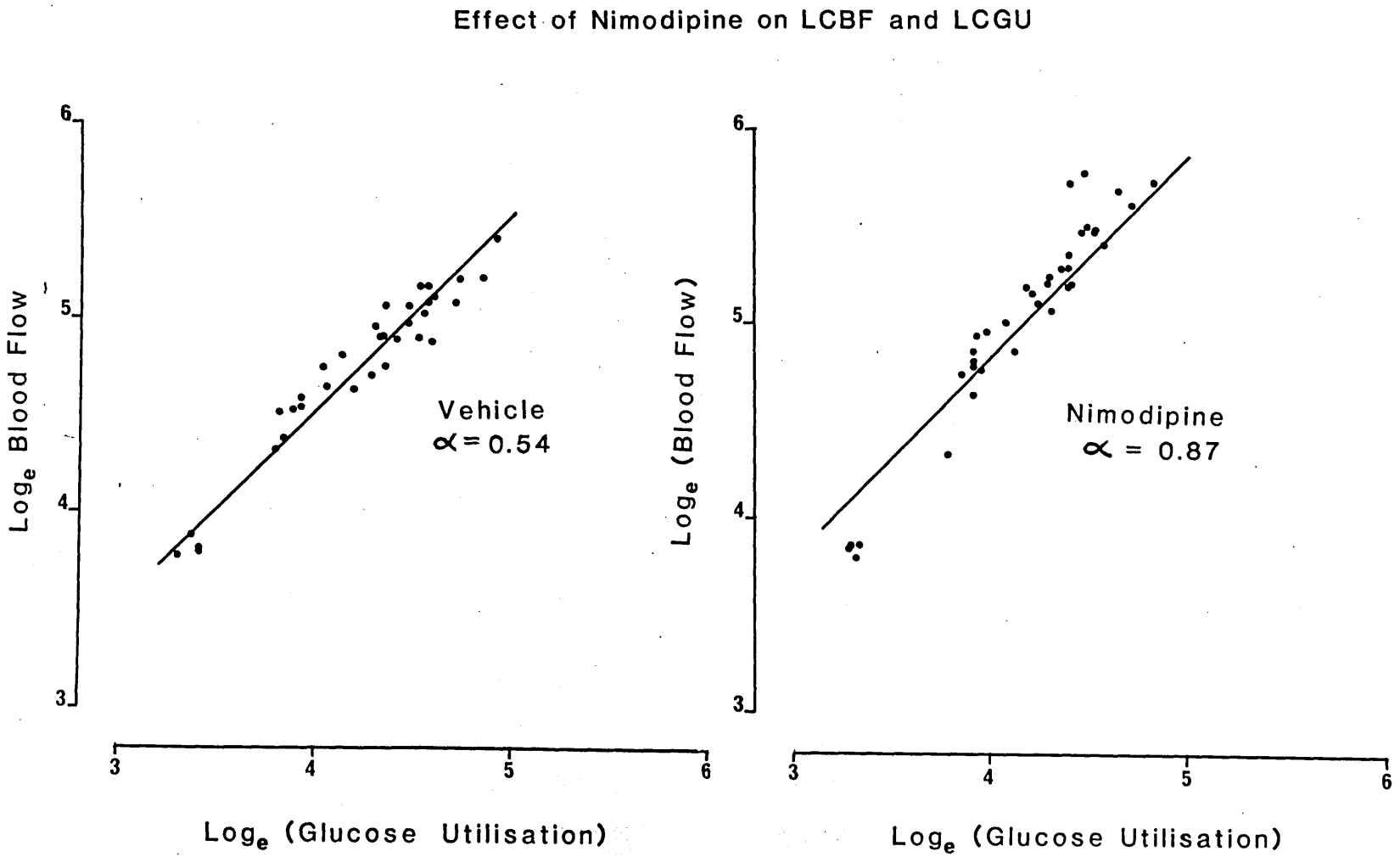


Figure 20. The relationship between the natural logarithms of mean LCBF and mean LCGU in control animals (left) and animals that received nimodipine (right). The intercepts of the lines (α) are indicated. For details of the rationale for the use of natural logarithms, see McCulloch et al. (1982).

3. Effects of Nimodipine on Local CBF and Neuropathological Patterns in Anaesthetised Rats following MCA Occlusion

3.1 Effect of pre-treatment with nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) on local CBF in rats following MCA occlusion.

3.1.1 General

There were no significant changes in the blood gas tensions, plasma glucose levels and arterial pH between vehicle and nimodipine pre-treated groups (Table 22). The PCO_2 varied between 39 $\pm$ 1 to 40 $\pm$ 1 (mm Hg), PO_2 varied between 131 $\pm$ 3 to 128 $\pm$ 8 (mm Hg), and pH between 7.47 $\pm$ 0.01 to 7.44 $\pm$ 0.01; plasma glucose levels (mM) varied between 9 $\pm$ 1 to 10 $\pm$ 1. The MABP (mm Hg) decreased with nimodipine infusion by 8% (Table 22); this failed to reach the accepted significance level.

3.1.2 Results

A. Control animals.

The level of local CBF in 34 anatomically discrete regions ipsilateral and contralateral to the MCA occlusion are shown in Table 23. Visual inspection of the autoradiograms from both vehicle and nimodipine groups showed marked reductions in local CBF in the hemisphere ipsilateral to the MCA occlusion as compared to the contralateral hemisphere. Marked reductions in local CBF were observed throughout the cerebral cortices ipsilateral to MCA occlusion: the visual cortex (by 61%), auditory cortex (by 73%), parietal cortex (by 71%), sensory-motor cortex (by 78%) and frontal cortex (by 75%).

The level of blood flow was reduced massively (by 92%) in the caudate nucleus ipsilaterally when compared to the

contralateral side of the lesion. Modest reductions (range 13-30%; average 22%) were observed in the ipsilateral, mediodorsal and ventrolateral thalamus, medial geniculate body, hippocampus, dentate gyrus, amygdala, nucleus accumbens, substantia nigra compacta, and genu. The level of blood flow in other regions subjected to MCA occlusion were generally similar to those compared to the contralateral side of the lesions (habenula, hypothalamus, septal nucleus, vestibular nucleus, cochlear nucleus, superior olive, inferior colliculus, pons, cerebellum hemisphere, cerebellum nuclei, cerebellum white, corpus callosum, and internal capsule). Some regions in the hemisphere contralateral to the occluded vessel showed increased CBF levels. These regions included globus pallidus and substantia nigra (reticulata).

B. Nimodipine pre-treated groups.

The pattern of local CBF levels in regions of the CNS were similar to those animals receiving vehicle when comparisons are made between the ipsilateral and contralateral sides to the occluded vessels (Table 23). The administration of nimodipine modified the pattern of local CBF disturbances in most cortical and subcortical regions of the brain examined compared with the corresponding sites during infusion of the vehicle that were observed in both contralateral and ipsilateral sides.

Comparison of LCBF in contralateral hemisphere in rats with MCA occlusion, pre-treated with nimodipine or vehicle.

Nimodipine pre-treatment at a dose of 1 $\mu g\ kg^{-1} min^{-1}$ increased the local CBF significantly in 26 of 34 regions of the contralateral hemisphere (Table 23), particularly in the

neocortical areas, including visual cortex, auditory cortex, parietal cortex, sensory-motor cortex and frontal cortex (range 76-96%; average 86%). Modest increases in the local CBF occurred in some subcortical regions; for example, thalamus mediodorsal (by 56%), hippocampus (by 60%), amygdala (by 54%), globus pallidus and substantia nigra compacta (by 36%). The CBF levels were unaltered in regions which included the cerebellar white, corpus callosum, genu of corpus callosum, and internal capsule.

Comparison of LCBF in ipsilateral hemisphere in rats with MCA occlusion, pre-treated with nimodipine or vehicle.

The ipsilateral reductions in the local blood flow observed in vehicle groups were significantly less pronounced with nimodipine (1 $\mu g\ kg^{-1}min^{-1}$) pre-treatment (Table 23, Figure 21). The main areas in which the reduction in flow was minimised were the cortical regions (visual cortex, auditory cortex, parietal cortex, sensory-motor cortex and frontal cortex). This pattern of increasing the flow in the hypoperfused regions (ipsilateral to the lesion) was also observed in some subcortical regions; for example, nucleus accumbens, dentate gyrus, hippocampus, thalamus (mediodorsal), substantia nigra (compacta), amygdala, and medial geniculate body. By contrast, there were no changes in the distribution of blood flow in the centre of the ischaemic lesion such as the caudate nucleus. In addition, nimodipine pre-treatment did not produce any changes in the level of blood flow in the genu of corpus callosum.

Effect of nimodipine pre-treatment on the frequency distribution of CBF following MCA occlusion.

The frequency distribution of the level of CBF was measured bilaterally (ipsilateral and contralateral to MCA occlusion in animals receiving vehicle or nimodipine) in parietal cortex, auditory cortex, sensory-motor cortex and caudate nucleus (Figs.26,27) (Tables 24-27). These areas were selected to assess the influence of nimodipine pre-treatment in regions outside the centre of ischaemia (cortical areas) and the core of ischaemic damage (caudate nucleus) in rats following MCA occlusion. The analysis of data was performed according to the method of measuring the CBF frequency distribution described previously (Chapter II, Section 2.3.2).

Both in nimodipine and vehicle groups there were significant differences between the parietal cortex, auditory cortex, sensory-motor cortex and caudate nucleus side ipsilateral to the MCA occlusion when compared to the contralateral side (Tables 24-27). In animals receiving vehicle, the area of auditory cortex with CBF less than or equal to 25 ml $100g^{-1}$ min^{-1} was 10 $\pm$ 2% (contralateral) and 20 $\pm$ 3% (ipsilateral), and in nimodipine pre-treated animals the area of auditory cortex with CBF less than or equal to 25 ml $100g^{-1}min^{-1}$ was 8 $\pm$ 1% (contralateral) and 3 $\pm$ 1% (ipsilateral) (Table 24). A similar pattern of redistribution of the level of blood flow was observed in the other cortical areas examined (see Tables 25 and 26). By contrast, nimodipine pre-infusion failed to modify the level of blood flow in the ischaemic caudate nucleus, when comparison was made between ipsilateral (vehicle) to the ipsilateral side to the ischaemic lesion when pre-treated with nimodipine (Table 27).

TABLE 22. PHYSIOLOGICAL VARIABLES FOR LCBF GROUP.

Variable	Vehicle n=5	Nimodipine n=5
$PaCO_2$ (mm Hg)	40 ± 1	39 ± 1
PaO_2 (mm Hg)	131 ± 3	128 ± 8
pH	7.47 ± 0.01	7.44 ± 0.01
Arterial Plasma Glucose (mM)	9 ± 1	10 ± 1
MABP (mm Hg)	111 ± 9	102 ± 6

Data are presented as mean ± SE. Measurements of the blood gases, pH and blood glucose were taken 10 min prior to the measurement of CBF and 20 min after commencement of the infusion of either vehicle or nimodipine. There are no significant differences.

TABLE 23. EFFECT OF NIMODIPINE (1 $\mu g\ kg^{-1} min^{-1}$) PRE-TREATMENT ON LCBF FOLLOWING MCA OCCLUSION.

Structure	Vehicle (n:5)		Nimodipine (n:5)	
	Contralateral	Ipsilateral	Contralateral	Ipsilateral
1. Visual cortex	135 ± 2	53 ± 5 **	265 ± 23 ++	123 ± 14 + **
2. Auditory cortex	156 ± 12	42 ± 5 **	289 ± 14 ++	96 ± 1 ++ **
3. Parietal cortex	147 ± 10	43 ± 5 **	259 ± 23 +	80 ± 6 + **
4. Sensory-motor cortex	135 ± 14	30 ± 3 **	246 ± 20 +	61 ± 9 + **
5. Frontal cortex	126 ± 8	31 ± 3 **	243 ± 23 +	61 ± 6 + **
6. Thalamus (MD)	129 ± 3	89 ± 5 **	202 ± 28	148 ± 15
7. Thalamus (VL)	111 ± 9	97 ± 3	151 ± 23	111 ± 13
8. Habenula	146 ± 13	137 ± 12	249 ± 20 +	249 ± 18 +
9. Medial geniculate body	149 ± 9	128 ± 4	275 ± 19 ++	242 ± 23 +
10. Lateral geniculate body	122 ± 14	110 ± 6	220 ± 24 +	181 ± 20 **
11. Hypothalamus	90 ± 6	90 ± 7	128 ± 15	131 ± 15
12. Hippocampus	141 ± 12	101 ± 3 *	225 ± 13 +	182 ± 18 + **
13. Dentate gyrus	110 ± 4	88 ± 4 **	192 ± 12 ++	147 ± 21 *
14. Amygdala	99 ± 12	78 ± 7	152 ± 12	103 ± 11 **
15. Septal nucleus	75 ± 5	77 ± 9	110 ± 10	112 ± 8
16. Caudate nucleus	124 ± 7	9 ± 1 **	199 ± 17 +	9 ± 3 **
17. Nucleus accumbens	125 ± 11	94 ± 10 *	185 ± 19	141 ± 15
18. Globus pallidus	65 ± 6	95 ± 8 **	101 ± 8	129 ± 11

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TABLE 23 (Contd.).

Structure	Vehicle (n:5)		Nimodipine (n:5)	
	Contralateral	Ipsilateral	Contralateral	Ipsilateral
19. Red nucleus	152 ± 9	147 ± 8	257 ± 14 ++	252 ± 13 ++
20. Substantia nigra (compacta)	114 ± 7	87 ± 6 **	196 ± 18 +	171 ± 14 ++ **
21. Substantia nigra (reticulata)	87 ± 8	100 ± 9	154 ± 16	167 ± 14 + **
22. Subthalamic nucleus	131 ± 4	124 ± 8	242 ± 32	210 ± 27
23. Vestibular nucleus	157 ± 8	148 ± 7	296 ± 11 ++	279 ± 9 ++
24. Cochlear nucleus	163 ± 10	166 ± 8	291 ± 9 ++	293 ± 14 ++
25. Superior olive	169 ± 10	167 ± 10	289 ± 14 ++	290 ± 10 ++
26. Inferior colliculus	195 ± 15	195 ± 14	313 ± 6 ++	309 ± 13 ++
27. Superior colliculus	122 ± 10	142 ± 8 **	258 ± 16 ++	250 ± 11 ++
28. Pons	79 ± 5	78 ± 4	137 ± 14 +	134 ± 15 +
29. Cerebellar hemisphere	73 ± 5	73 ± 5	133 ± 11 +	130 ± 10 +
30. Cerebellar nuclei	140 ± 7	145 ± 7	276 ± 14 ++	277 ± 14 ++
31. Cerebellar white	35 ± 3	36 ± 2	42 ± 2	43 ± 2
32. Corpus callosum	36 ± 3	36 ± 3	42 ± 3	43 ± 3
33. Genu	32 ± 4	24 ± 2	40 ± 2	24 ± 3 **
34. Internal capsule	37 ± 4	36 ± 3	41 ± 3	41 ± 3

Data are presented as mean ± SE. *$p < 0.05$; **$p < 0.01$, significance of difference between data on ipsilateral and contralateral sides within the same animals. +$p < 0.05$; ++$p < 0.01$, significance of difference between data for comparison between vehicle and nimodipine treated animals.

TABLE 24. AUDITORY CORTEX : PER CENT AREA/CUMULATIVE FLOW RELATIONSHIP.

	Vehicle (n=5)		Nimodipine (n=5)	
Cumulative Flow	Contralateral	Ipsilateral	Contralateral	Ipsilateral
0 - 25	10 ± 2	20 ± 3$^{\phi}$	8 ± 1	3 ± 1$^{\phi *}$
25 - 50	15 ± 4	55 ± 4$^{\phi}$	12 ± 1	22 ± 3$^{\phi *}$
50 - 75	25 ± 6	76 ± 5$^{\phi}$	14 ± 1	34 ± 2$^{\phi *}$
75 - 100	37 ± 9	85 ± 5$^{\phi}$	18 ± 2	48 ± 3$^{\phi *}$
100 - 125	53 ± 10	93 ± 3$^{\phi}$	23 ± 2*	60 ± 4$^{\phi *}$
125 - 150	69 ± 10	97 ± 1$^{\phi}$	31 ± 5*	68 ± 6$^{\phi *}$
150 - 175	79 ± 9	99 ± 1	41 ± 5*	78 ± 7$^{\phi *}$
175 - 200	87 ± 6	99 ± 1	53 ± 6*	83 ± 6$^{\phi *}$

Values are mean ± SEM (the per cent of area at different flow levels in ml $100g^{-1}min^{-1}$). $\phi p < 0.05$ Student's paired t-test for MCA occlusion, $*p < 0.05$ Student's unpaired t-test for nimodipine.

TABLE 25. PARIETAL CORTEX : PER CENT AREA/CUMULATIVE FLOW RELATIONSHIP.

	Vehicle (n=5)		Nimodipine (n=5)	
Cumulative Flow	Contralateral	Ipsilateral	Contralateral	Ipsilateral
0 - 25	8 ± 1	34 ± 5$^{\phi}$	5 ± 2	17 ± 5
25 - 50	16 ± 1	72 ± 5$^{\phi}$	8 ± 2*	44 ± 5$^{\phi *}$
50 - 75	29 ± 2	85 ± 3$^{\phi}$	14 ± 4*	69 ± 2$^{\phi *}$
75 - 100	47 ± 3	93 ± 3$^{\phi}$	24 ± 8*	82 ± 3$^{\phi *}$
100 - 125	64 ± 3	97 ± 1$^{\phi}$	45 ± 9*	89 ± 3$^{\phi}$
125 - 150	81 ± 4	98 ± 1$^{\phi}$	49 ± 11*	93 ± 3$^{\phi}$
150 - 175	90 ± 4	99 ± 1$^{\phi}$	57 ± 11*	95 ± 2$^{\phi}$
175 - 200	95 ± 3	99 ± 1	65 ± 11*	97 ± 1$^{\phi}$

Values are mean ± SEM (the per cent of area at different flow levels in ml $100g^{-1}min^{-1}$). $\phi p < 0.05$ Student's paired t-test for MCA occlusion, $*p < 0.05$ Student's unpaired t-test for nimodipine.

TABLE 26. SENSORY-MOTOR CORTEX : PER CENT AREA/CUMULATIVE FLOW RELATIONSHIP.

	Vehicle (n=5)		Nimodipine (n=5)	
Cumulative Flow	Contralateral	Ipsilateral	Contralateral	Ipsilateral
0 - 25	13 ± 2	47 ± 4$^{\phi}$	7 ± 1*	29 ± 7$^{\phi}$
25 - 50	20 ± 1	78 ± 3$^{\phi}$	11 ± 1*	58 ± 7$^{\phi *}$
50 - 75	30 ± 2	91 ± 2$^{\phi}$	14 ± 1*	75 ± 5$^{\phi *}$
75 - 100	48 ± 6	96 ± 1$^{\phi}$	19 ± 2*	87 ± 1$^{\phi *}$
100 - 125	66 ± 8	98 ± 1$^{\phi}$	25 ± 3*	93 ± 1$^{\phi *}$
125 - 150	84 ± 1	99 ± 1$^{\phi}$	42 ± 11*	96 ± 1$^{\phi *}$
150 - 175	93 ± 4	99 ± 1	48 ± 5*	97 ± 1$^{\phi *}$
175 - 200	97 ± 1	99 ± 1	60 ± 11*	98 ± 1$^{\phi *}$

Values are mean ± SEM (the per cent of area at different flow levels in ml $100g^{-1}min^{-1}$). $\phi p < 0.05$ Student's paired t-test for MCA occlusion, $*p < 0.05$ Student's unpaired t-test for nimodipine.

TABLE 27. CAUDATE NUCLEUS : PER CENT AREA/CUMULATIVE FLOW RELATIONSHIP.

	Vehicle (n=5)		Nimodipine (n=5)	
Cumulative Flow	Contralateral	Ipsilateral	Contralateral	Ipsilateral
0 - 25	1 ± 1	60 ± 7$^{\phi}$	1 ± 1	32 ± 8$^{\phi}$
25 - 50	2 ± 1	75 ± 5$^{\phi}$	1 ± 1	55 ± 1$^{\phi}$
50 - 75	18 ± 9	91 ± 2$^{\phi}$	1 ± 1	71 ± 8$^{\phi}$
75 - 100	41 ± 14	98 ± 1$^{\phi}$	6 ± 4*	80 ± 7$^{\phi}$
100 - 125	67 ± 13	99 ± 1	16 ± 8*	87 ± 5$^{\phi}$
125 - 150	84 ± 9	99 ± 1	33 ± 14*	94 ± 3$^{\phi}$
150 - 175	92 ± 5	99 ± 1	55 ± 0	97 ± 3$^{\phi}$
175 - 200	97 ± 1	100 ± 0	72 ± 12	99 ± 1

Values are mean ± SEM (the per cent of area at different flow levels in ml $100g^{-1}min^{-1}$). $\phi p < 0.05$ Student's paired t- test for MCA occlusion, $*p \leq 0.05$ Student's unpaired t-test for nimodipine.

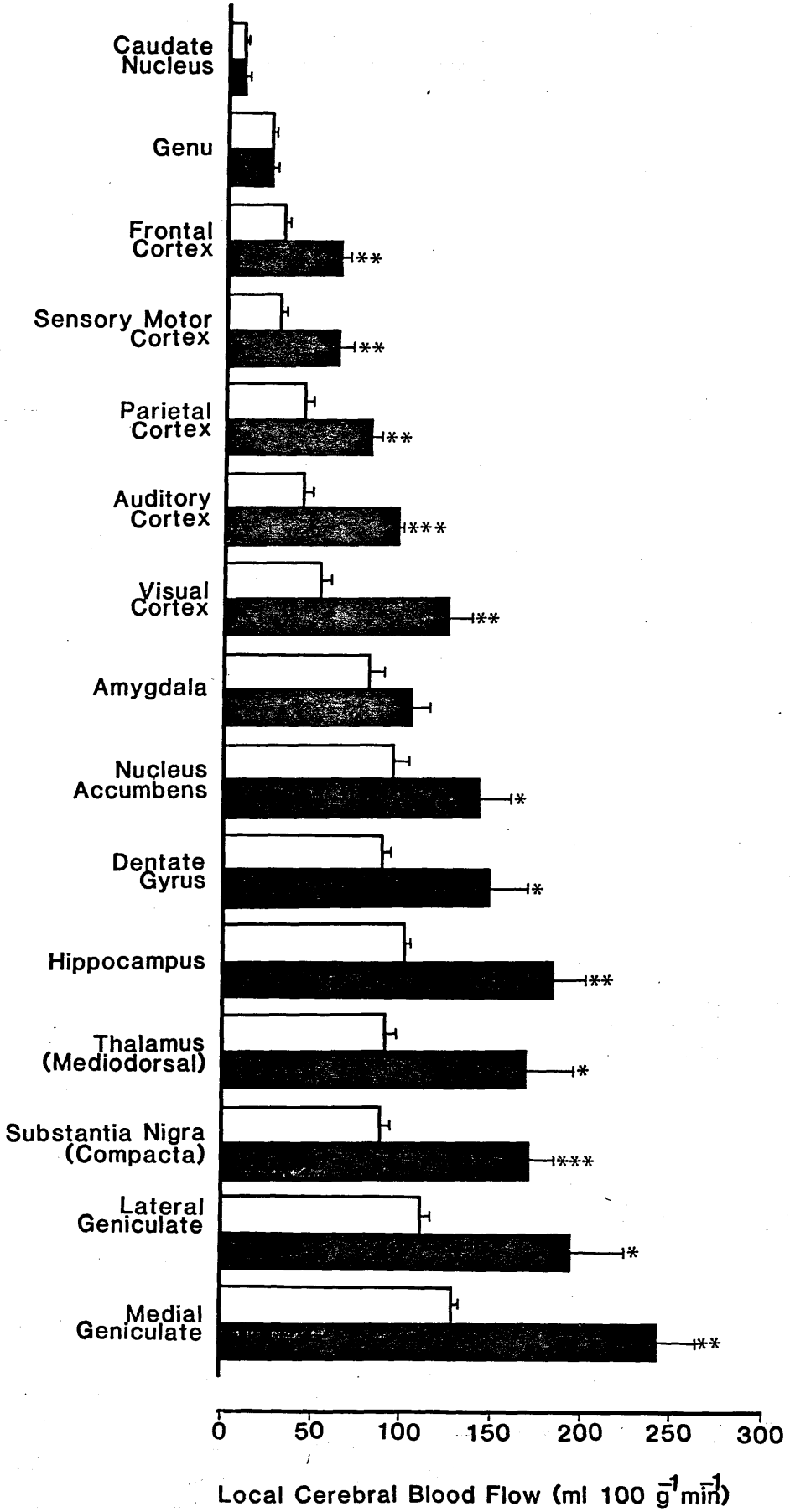

Figure 21. The white histograms represent brain regions with hypoperfusion ipsilateral to MCA occlusion after infusion of vehicle. The solid histograms are for the corresponding ipsilateral areas with nimodipine pre-treatment (n=5 for each group). The values are mean ± SE; *$p<0.05$; **$p<0.01$; ***$p<0.001$, using unpaired Student's t-test.

3.2 Effect of pre-treatment with nimodipine (2 $\mu g\ kg^{-1}min^{-1}$) on local CBF in rats following MCA occlusion.

3.2.1 General

There were no significant changes in the blood gas tensions, plasma glucose levels and arterial pH between saline and nimodipine pre-treated groups (Table 28). The MABP (mm Hg) decreased with nimodipine (2 $\mu g\ kg^{-1}min^{-1}$) by 16% (Table 28).

3.2.2 Results

A. Control rats (receiving saline) studied after MCA occlusion.

The level of local CBF in 34 anatomically discrete regions ipsilateral and contralateral to the MCA occlusion are shown in Table 29. Marked reductions in local CBF were observed throughout the cerebral cortices ipsilateral to MCA occlusion, in the visual cortex by 52%, parietal cortex by 75%, sensory-motor cortex by 72%, and frontal cortex by 72%.

The level of blood flow was reduced massively (by 86%) in the caudate nucleus ipsilaterally when compared to the contralateral side of the MCA occlusion. Modest reductions (range 11-44%; average 28%) were observed in the ipsilateral thalamus (mediodorsal), thalamus (ventrolateral), medial geniculate body, lateral geniculate body, hippocampus, dentate gyrus, amygdala, septal nucleus, nucleus accumbens, substantia nigra (pars compacta) and genu of the corpus callosum. The levels of blood flow in other ipsilateral brain regions were generally similar to those in the hemisphere contralateral to the lesion. These regions were the habenula, hypothalamus, vestibular nucleus, cochlear nucleus, superior olive, inferior colliculus, pons,

cerebellum hemisphere, cerebellum nuclei, cerebellum white, corpus callosum and internal capsule.

B. Animals pre-treated with nimodipine before MCA occlusion.

When comparisons were made of the effects of nimodipine in contralateral hemisphere with the data from control rats, it was found that nimodipine pre-treatment at a dose of 2 $\mu g\ kg^{-1}\ min^{-1}$ increased the local CBF significantly in 10 regions (Table 29). These included the auditory cortex (by 37%), parietal cortex (by 48%), sensory motor-cortex (by 50%) and frontal cortex (by 50%). Increases in the level of CBF were also observed in some subcortical regions; for example, medial geniculate body (by 36%), lateral geniculate body (by 40%), hippocampus (by 44%), dentate gyrus (by 35%), caudate nucleus (by 44%) and nucleus accumbens (by 51%). When the hemisphere ipsilateral to occlusion was studied, it was seen that the administration of nimodipine modified the pattern of local CBF disturbances in most of the cortical and subcortical regions affected by MCA occlusion. Compared with the controls, the reduction in the local CBF observed after pre-treatment with nimodipine was significantly less; that is, the LCBF in the ipsilateral hemisphere was higher than after occlusion in untreated animals (Table 29). The main areas in which the reduction in flow was minimised were the cortical regions, auditory and parietal cortices; for example, in parietal cortex the value without pre-treatment was 39 $\pm$ 6, and after pre-treatment was 97 $\pm$ 10, compared with value in contralateral hemisphere in untreated rats (156 $\pm$ 10). The values observed were, untreated 75% and treated 38%, and the latter was 58% of the value of the contralateral hemisphere in treated rats.

TABLE 28. PHYSIOLOGICAL VARIABLES OF NIMODIPINE (2 μg $kg^{-1}min^{-1}$) PRE-TREATMENT FOLLOWING MCA OCCLUSION.

Variable	Control n=9	Nimodipine n=7
PCO_2 (mm Hg)	33.4 ± 1.5	36 ± 1
PO_2 (mm Hg)	116.1 ± 4.3	120 ± 8
pH	7.44 ± 0.02	7.43 ± 0.01
Plasma glucose (mM)	10.8 ± 0.9	11 ± 0.02
MABP (mm Hg)	108 ± 4	91 ± 4*

Data presented were measured 5 min before the determination of CBF. Values are mean ± SE. $p<0.05$ Student's t-test.

TABLE 29. EFFECT OF NIMODIPINE (2 μg $kg^{-1}min^{-1}$) PRE-TREATMENT ON LCBF FOLLOWING MCA OCCLUSION.

	Control n=9		Nimodipine n=7	
Structure	Contralateral	Ipsilateral	Contralateral	Ipsilateral
Visual cortex	142 ± 11	69 ± 12 **	202 ± 24	120 ± 16 **
Auditory cortex	164 ± 12	44 ± 5 **	224 ± 14 +	114 ± 15 ** ++
Parietal cortex	156 ± 10	39 ± 6 **	231 ± 18 +	97 ± 10 ** ++
Sensory-motor cortex	143 ± 9	41 ± 7 **	214 ± 17 +	54 ± 2 **
Frontal cortex	137 ± 9	39 ± 6 **	200 ± 21 +	55 ± 1 **
Thalamus (mediodorsal)	122 ± 8	87 ± 7 *	172 ± 17	145 ± 13 * ++
Thalamus (ventrolateral)	95 ± 7	78 ± 7 **	136 ± 16	109 ± 14 *
Habenula	161 ± 7	159 ± 7	213 ± 18	207 ± 17
Medial geniculate body	157 ± 9	125 ± 10 **	214 ± 16 +	184 ± 16 ** +
Lateral geniculate body	125 ± 8	105 ± 8 **	175 ± 15 +	149 ± 13 ** +
Hypothalamus	85 ± 7	83 ± 6	109 ± 10	107 ± 10
Hippocampus	119 ± 7	93 ± 7 **	171 ± 9 ++	141 ± 10 ** ++
Dentate gyrus	109 ± 6	85 ± 7 **	147 ± 11 +	127 ± 10 ** +
Amygdala	102 ± 9	79 ± 8 *	145 ± 19	111 ± 15 *
Septal nucleus	81 ± 7	72 ± 7 *	101 ± 9	92 ± 9 *
Caudate nucleus	127 ± 8	18 ± 3 **	183 ± 9 ++	24 ± 4 **
Nucleus accumbens	120 ± 8	77 ± 9 **	181 ± 13 ++	121 ± 5 ** +
Globus pallidus	59 ± 4	74 ± 4 **	78 ± 10	108 ± 14 *

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TABLE 29 (Contd.).

Structure	Control n=9		Nimodpine n=7	
	Contralateral	Ipsilateral	Contralateral	Ipsilateral
Red nucleus	154 ± 6	150 ± 8	201 ± 18	200 ± 18
Substantia nigra (compacta)	120 ± 4	103 ± 5 **	144 ± 14	123 ± 12
Substantia nigra (reticulata)	85 ± 6	100 ± 6 **	89 ± 8	121 ± 14 *
Subthalamic nucleus	133 ± 10	127 ± 10	178 ± 23	162 ± 19
Vestibular nucleus	182 ± 7	180 ± 6	219 ± 16	219 ± 17
Cochlear nucleus	190 ± 4	191 ± 5	233 ± 19	227 ± 18
Superior olive	180 ± 5	183 ± 4	206 ± 13	204 ± 13
Inferior colliculus	202 ± 7	203 ± 7	263 ± 24	259 ± 25
Superior colliculus	146 ± 5	163 ± 5 **	157 ± 12	202 ± 14
Pons	105 ± 6	104 ± 7	120 ± 11	121 ± 11
Cerebellum hemisphere	91 ± 6	93 ± 6	101 ± 8	101 ± 8
Cerebellum nuclei	175 ± 7	170 ± 9	205 ± 18	205 ± 19
Cerebellum white	42 ± 1	43 ± 2	51 ± 4	50 ± 3
Corpus callosum	41 ± 2	40 ± 2	48 ± 4	47 ± 4
Genu	41 ± 2	23 ± 2 **	48 ± 4	24 ± 3 **
Internal capsule	43 ± 2	41 ± 2	50 ± 5	49 ± 3

*$p<0.05$, **$p<0.01$, Student's paired t-test; significant difference between data on ipsilateral and contralateral sides within the same animal. +$p<0.05$, ++$p<0.01$, Student's unpaired t-test for comparison between control and nimodipine pre-treated animals. Local CBF (ml $100g^{-1}min^{-1}$) data are presented as mean ± SE, for grouped data within Bonferroni correction.

3.3 Effect of pre-treatment with nimodipine (1 $\mu g\ kg^{-1} min^{-1}$) on neuropathological patterns in rats following MCA occlusion.

3.3.1 General

There was not a significant difference between the blood gas tensions, plasma glucose levels and arterial pH between vehicle and nimodipine pre-treated groups (Table 30); PCO_2 range 39 ± 1 to 40 ± 1, PO_2 range 131 ± 5 to 140 ± 4, pH range 7.42 ± 0.01 to 7.44 ± 0.02, and plasma glucose levels - range 9 ± 1 to 10 ± 1.

In the animals that received vehicle there was not a significant alteration in the MABP values as compared with the base line level (Table 30). However, in nimodipine treated animals, throughout the infusion period of the drug (Table 30), there was a small reduction (7%) in the MABP, which did not reach a significant level when compared with the base line value.

3.3.2 Effect on the neuropathological pattern

The amount of ischaemic damage in the hemisphere, cortex and caudate nucleus was measured according to Graham et al. (1984) (see Chapter II, Section 5). The area of ischaemic damage in the hemisphere, cortex and caudate nucleus in control and nimodipine pre-treated animals is shown in Figures 22-24. There was extensive ischaemic damage in the ipsilateral neostriatum of all the animals with MCA occlusion. Comparisons of the distribution of the ischaemic lesion at each stereotactic level showed that the damage was less extensive in rats treated with nimodipine as compared with the control rats (Figures 22, 23). The volumes of ischaemic damage in the hemisphere, cortex and caudate nucleus in vehicle receiving

animals and nimodipine pre-treated animals after MCA occlusion are shown in Figure 25. The volume of hemispheric damage was reduced from 47 $\pm$ 7 mm^3 to 31 $\pm$ 3 mm^3 in nimodipine pre-treated rats. The volume of cortical damage was reduced from 26 $\pm$ 6 mm^3 with vehicle to 16 $\pm$ 3 mm^3 with nimodipine.

Although nimodipine pre-treatment produced a marked reduction in the volume of ischaemia, this just failed to reach the accepted significance level when treated by Student's t-test. This may be explained by the fact that there was considerable inter-animal variability of presence of ischaemic damage when the middle cerebral artery occlusion was performed. However, a trend towards a beneficial effect of the drug in minimising the volume of ischaemic infarct can be observed (Figure 25). By contrast, there was not a significant difference in the effect of nimodipine on the volume of ischaemic damage in the caudate nucleus which was, with nimodipine, 13 $\pm$ 2 mm^3 compared with 15 $\pm$ 2 mm^3 with vehicle (Figure 25).

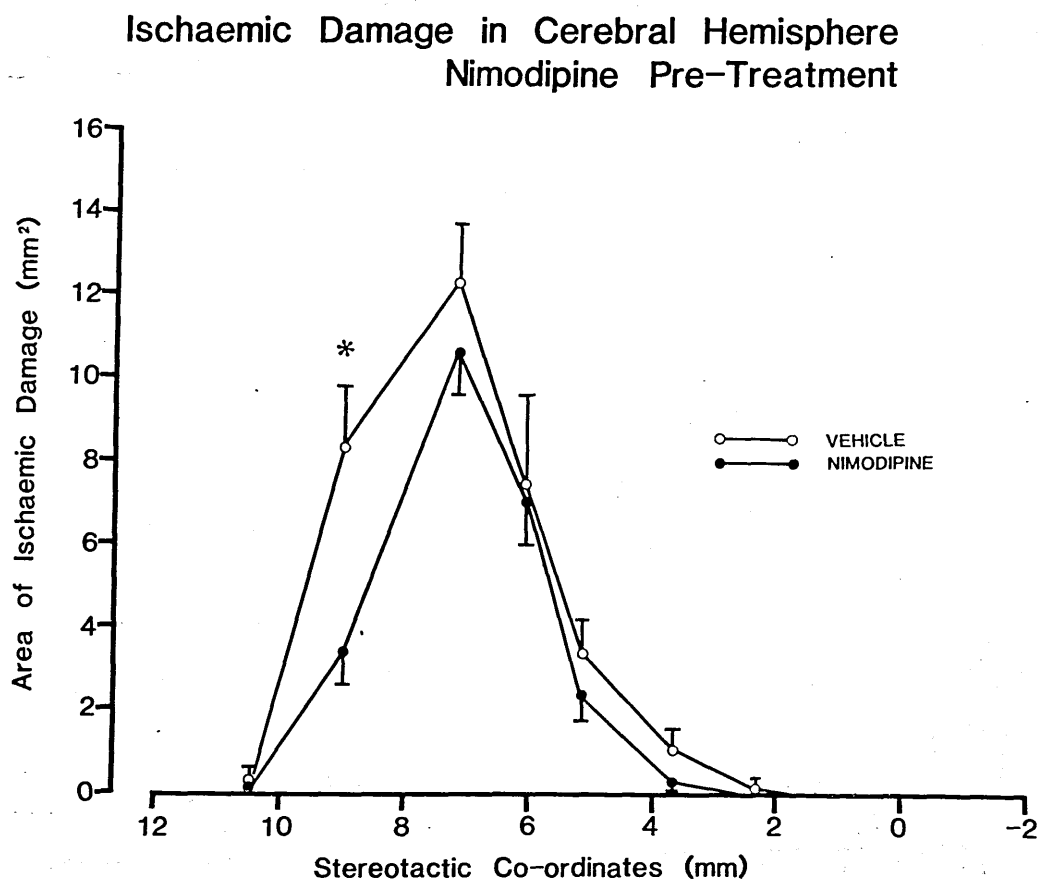
TABLE 30. PHYSIOLOGICAL VARIABLES FOR NEUROPATHOLGICAL STUDY GROUP.

	Vehicle (n=8)			Nimodipine (n=8)		
Variable	Base Line	60 Min	120 Min	Base Line	60 Min	120 Min
$PaCO_2$ (mm Hg)	40 ± 1	39 ± 1	40 ± 1	39 ± 1	40 ± 1	39 ± 1
PaO_2 (mm Hg)	132 ± 1	137 ± 6	131 ± 5	140 ± 3	140 ± 4	136 ± 4
pH	7.43 ± 0.01	7.44 ± 0.01	7.44 ± 0.01	7.44 ± 0.02	7.42 ± 0.01	7.44 ± 0.01
Arterial plasma glucose (mM)	9 ± 0.4	10 ± 1	9 ± 1	9 ± 1	10 ± 1	10 ± 1
MABP (mm Hg)	105 ± 4	104 ± 4	102 ± 5	111 ± 6	103 ± 5	103 ± 5

Data, which are presented as mean ± SE, are those immediately prior to MCA occlusion (base line) and at 60 minutes and 120 minutes after occlusion.

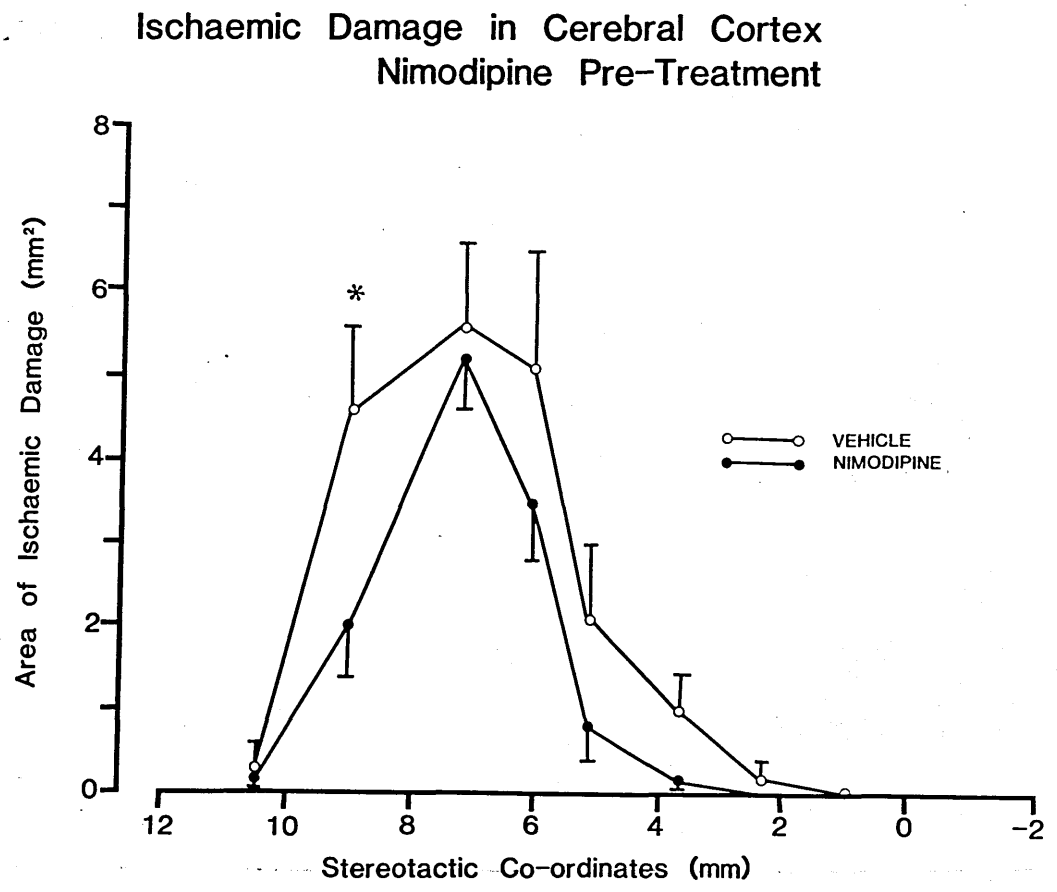
There are no significant differences between vehicle and nimodipine treatments.

Figure 22.



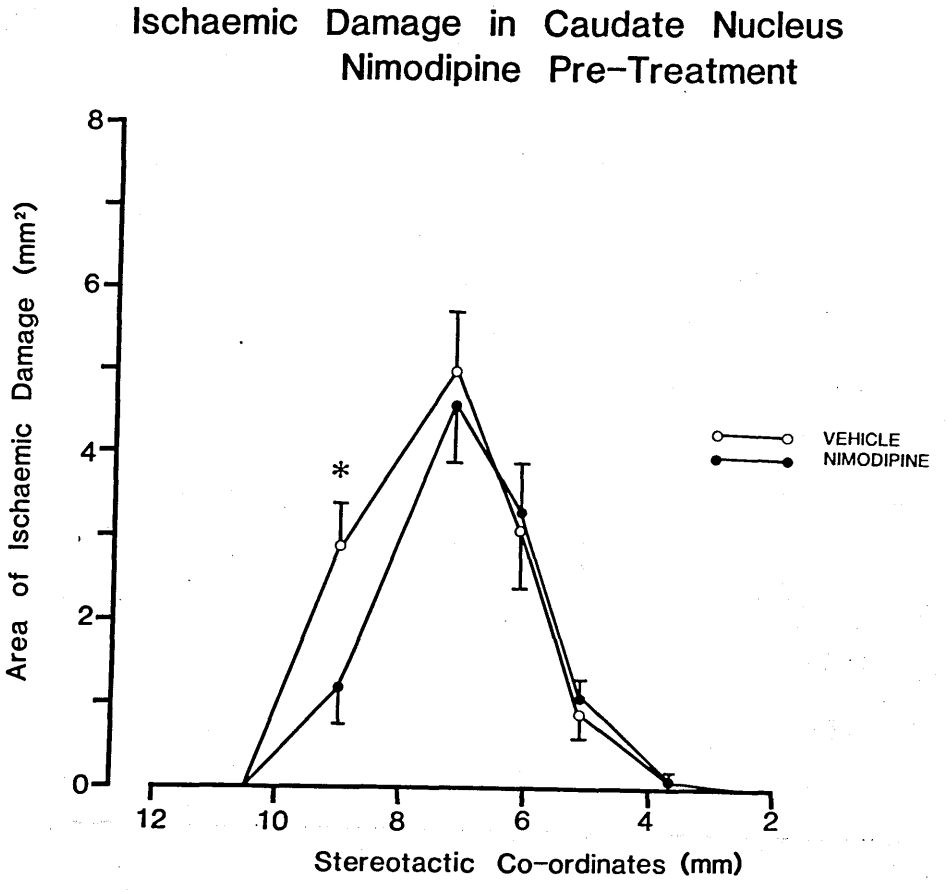
Amount of ischaemic damage (mm^2) at different stereotactically determined positions in the cerebral hemisphere for vehicle (control n=8) and nimodipine pre-treated (n-8) animals; unpaired Student's t-test; M $\pm$ SE; *$p < 0.05$.

Figure 23.



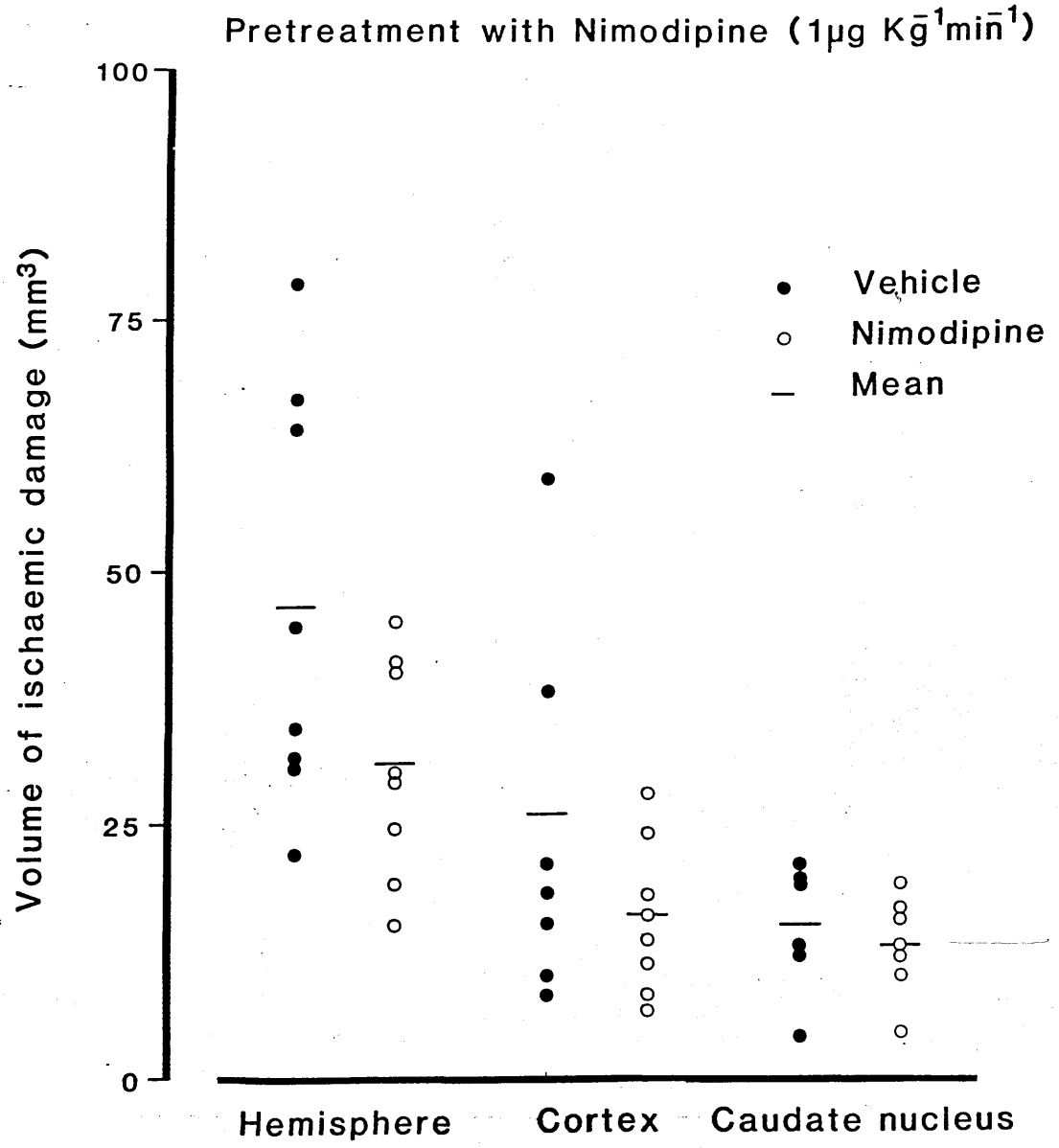
Amount of ischaemic damage (mm^2) at different stereotactically determined positions in the cerebral cortex for vehicle (control n=8) and nimodipine pre-treated (n=8) animals. Unpaired Student's t-test; $M \pm SE$; $*p < 0.05$.

Figure 24.



Amount of ischaemic damage (mm^2) at different stereotactically determined positions in the caudate nucleus for vehicle (control n=8) and nimodipine pre-treated (n=8) animals. Unpaired Student's t-test; M $\pm$ SE; *$p < 0.05$.

Figure 25.



Volume of ischaemic damage (mm^3) in each animal pre-treated with nimodipine (1 μg $kg^{-1}min^{-1}$; open circles, n=8) compared with vehicle (control; solid circles, n=8).

3.4 Effect of post-treatment with nimodipine (1 $\mu g\ kg^{-1} min^{-1}$) on local CBF in rats following MCA occlusion.

3.4.1 General

The cardiovascular and respiratory status of the control and the nimodipine treated animals is shown in Table 31. The MABP at the time of CBF measurement (i.e., 30 min after nimodipine infusion was started) was significantly lower in the drug treated group than in the control. Otherwise, there was not a statistically significant difference between the two groups.

3.4.2 Effect of nimodipine on local CBF after MCA occlusion.

The levels of CBF in 35 neuroanatomically defined brain areas after 35 min of ischaemia are shown in Table 32. Both in the control and the drug treated groups, marked reductions in CBF were observed throughout the ipsilateral neocortex and the lateral neocortex, and the lateral neostriatum. There were also significant reductions in the ipsilateral cingulate cortex, nucleus accumbens, septal nucleus, ventral thalamus, amygdala, and medial neostriatum. Increases in CBF were observed in the ipsilateral globus pallidus and substantia nigra, but these increases achieved statistical significance only in the nimodipine treated animals. In the majority of the brain structures, including brain stem and cerebellum, CBF values were symmetrical.

The administration of nimodipine, initiated five minutes after MCA occlusion, did not modify the pattern of CBF disturbance in the territory of the occluded vessel, nor in any adjacent or distant area. The values of CBF both ipsilateral and contralateral to MCA occlusion were similar in the vehicle and nimodipine treated groups.

TABLE 31. PHYSIOLOGICAL VARIABLES FOR INVESTIGATION OF CBF AFTER NIMODIPINE (1 ug $kg^{-1}min^{-1}$) ADMINISTRATION.

Variable	Vehicle	Nimodipine
PCO_2	40.0 ± 1.3	39.2 ± 0.7
PO_2	153 ± 5	158 ± 7
pH	7.43 ± 0.03	7.41 ± 0.02
Plasma glucose	8.6 ± 0.5	9.8 ± 1.0
MABP	120 ± 7	95 ± 3*

Data are presented as mean ± SE (n=5) at the time of CBF measurement.

*$p < 0.02$ for the comparison between vehicle and nimodipine (Student's t-test).

TABLE 32. EFFECT OF NIMODIPINE (1 $\mu g\ kg^{-1} min^{-1}$) ON CBF AFTER MIDDLE CEREBRAL ARTERY OCCLUSION.

	Vehicle (n=5)		Nimodipine (n=5)	
Structure	Contralateral	Ipsilateral	Contralateral	Ipsilateral
Visual cortex	136 ± 6	58 ± 8*	141 ± 10	63 ± 8*
Auditory cortex	157 ± 11	53 ± 5*	172 ± 30	53 ± 5*
Parietal cortex	133 ± 12	36 ± 4*	143 ± 14	37 ± 5*
Sensory-motor cortex	131 ± 17	34 ± 2*	146 ± 14	34 ± 1*
Frontal cortex	138 ± 19	39 ± 4*	141 ± 11	39 ± 3*
Posterior cingulate cortex	119 ± 8	93 ± 4*	136 ± 13	117 ± 13*
Anterior cingulate cortex	119 ± 11	87 ± 9*	119 ± 14	89 ± 7*
Thalamus (mediodorsal nucleus)	109 ± 14	90 ± 10	99 ± 8	84 ± 7
Thalamus (ventrolateral nucleus)	103 ± 10	87 ± 9*	95 ± 5	85 ± 6*
Lateral habenula	146 ± 18	143 ± 16	129 ± 9	127 ± 8
Medial geniculate body	121 ± 10	102 ± 6	114 ± 7	113 ± 8
Lateral geniculate body	109 ± 11	105 ± 9	101 ± 7	98 ± 5
Hypothalamus	80 ± 6	79 ± 7	77 ± 5	78 ± 5
Hippocampus (molecular layer	109 ± 7	114 ± 7	117 ± 6	95 ± 3
Dentate gyrus	102 ± 5	103 ± 6	102 ± 6	89 ± 1
Amygdala	78 ± 7	60 ± 8	81 ± 4	68 ± 4
Septal nucleus	85 ± 5	78 ± 5	88 ± 7	74 ± 5
Caudate nucleus	119 ± 16	11 ± 4	113 ± 6	12 ± 3
Nucleus accumbens	118 ± 16	86 ± 9	114 ± 10	74 ± 10

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TABLE 32 (Contd.).

Structure	Vehicle (n=5)		Nimodipine (n=5)	
	Contralateral	Ipsilateral	Contralateral	Ipsilateral
Globus pallidus	84 ± 22	105 ± 23	68 ± 3	93 ± 6
Red nucleus	119 ± 14	113 ± 12	115 ± 6	107 ± 4
Substantia nigra	91 ± 44	101 ± 10	71 ± 4	80 ± 3
Subthalamic nucleus	130 ± 18	125 ± 18	115 ± 6	110 ± 8
Vestibular nucleus	160 ± 12	163 ± 12	148 ± 5	148 ± 4
Cochlear nucleus	178 ± 29	178 ± 30	150 ± 12	146 ± 10
Superior olivary nucleus	176 ± 19	178 ± 19	164 ± 11	169 ± 12
Inferior colliculus	181 ± 27	165 ± 23	167 ± 13	167 ± 15
Superior colliculus	128 ± 10	125 ± 8	120 ± 10	121 ± 8
Pons	86 ± 11	84 ± 9	75 ± 3	73 ± 2
Cerebellar hemisphere	74 ± 5	73 ± 5	65 ± 3	66 ± 3
Cerebellar nuclei	156 ± 13	160 ± 12	145 ± 5	145 ± 6
Cerebellar white matter	39 ± 4	39 ± 3	37 ± 3	38 ± 3
Corpus callosum	37 ± 3	37 ± 3	37 ± 2	36 ± 2
Genu of corpus callosum	36 ± 4	35 ± 4	36 ± 2	35 ± 2
Internal capsule	38 ± 3	37 ± 3	35 ± 2	36 ± 2

Data are presented as mean ± SE. There were no significant differences between vehicle and nimodipine treatment in any region (Student's t-test). *$p < 0.05$ for comparison between contralateral and ipsilateral sides in vehicle and nimodipine treated animals. CBF (ml $100g^{-1}min^{-1}$). Measurements were initiated 5 minutes after MCA occlusion.

CHAPTER IV

DISCUSSION

1. The Effects of Nimodipine Administration in LCBF in Anaesthetised Animals, and the Relationship between LCBF and LCGU in Conscious Animals.

1.1 The effects of nimodipine on LCBF in anaesthetised animals: relationship to arterial blood pressure.

The effects of nimodipine and other calcium antagonists on CBF have been extensively investigated in primates, dogs, cats, rats and man under conditions in which the cerebral circulation was intact (Harper et al. 1981; Kazda et al. 1982; Edvinsson et al. 1983; Haws et al. 1983; Haws and Heistad, 1983; McCalden et al. 1984) and with pre-existing cerebrovascular dysfunction (Hiffmeister et al. 1979; Gaab et al. 1982; Harris et al. 1982; Karasawa et al. 1982; Symon et al. 1982; Allen et al. 1983; Gygax and Wiernsperger, 1983; Faden et al. 1984; Newberg et al. 1984). No consensus has yet been established as to whether calcium antagonists, which are certainly potent dilators of cerebral vessels, do indeed increase CBF.

In the first part of this study three factors were addressed: the complicating actions of the drug on arterial pressure, the dose dependency, and regional heterogeneity in the cerebrovascular response.

The effects of nimodipine on MABP may have been an important factor on the cerebrovascular effects of the drug. Even with the lowest concentration of nimodipine studied (1 $\mu g\ kg^{-1} min^{-1}$) blood pressure was reduced modestly ($\sim$14%), and pronounced increases in CBF in rostral brain areas were observed. A four-fold increase in the infusion concentration reduced blood

pressure markedly (by 26%) and produced no further increase in CBF. The reason that increasing concentrations failed to provoke a further increase in CBF may have been because of the concomitant hypotension. This is analogous to the attenuation of hypercapnic cerebrovascular dilatation by hypotension (Harper and Glass, 1965). Although the interaction of two dilator stimuli (nimodipine and hypotension) may be a complicating factor in investigations in animals or humans with normal autoregulation, the effects of nimodipine in patients with impaired autoregulation, such as those with cerebrovascular disease (Overgaard and Tweed, 1974, 1983; Fitch et al. 1976; Kosnik and Hunt, 1976; Ledingham and Rajagopalan, 1979) may depend crucially on the effects of the drug on perfusion pressure. Thus, the hypotension produced by nimodipine may be deleterious in patients with impaired autoregulation or whose cerebral resistance vessels are already maximally dilated.

In any studies of cerebral blood flow, either in normal or in damaged brain, the choice of method of measurement has important implications. Each technique has particular advantages and disadvantages, which limits its use, even in specific experimental situations. The ^{133}Xenon clearance technique (Høedt-Rasmussen et al. 1966; Harper et al. 1972) should be used in primates, and not in dogs or cats in whom the brain is supplied both by the internal and external carotid arteries. Thus, it is difficult to avoid extracranial contamination. Multiple serial measurements can be obtained by this technique; in addition, metabolic studies are possible.

The ^{85}Krypton clearance technique (Harper et al. 1961; Ingvar and Lassen, 1962) allows repetitive measurements of flow of the cortical gray matter in all species. However, the

^{85}Krypton is a beta-emitting isotope and therefore requires surgical exposure of the cortex to avoid absorption of radioactivity by the skull. The microsphere trapping method (Marcus et al. 1976) can be used in any animal, and allows three to six flow measurements in multiple anatomically gross regions in the same animal. The major advantage of this technique is the possibility to measure the flow of the brain and other organs (for example, heart, kidney), and the same tissue sample which is used for flow measurements may also be assessed for tissue constituents (for example, water, electrolytes or biochemical substrates). However, the incomplete mixing or streaming of microspheres can produce artefacts. The hydrogen clearance technique (Pasztor et al. 1973) permits repeated flow measurements at multiple sites, but requires insertion of electrodes into the brain, and a traumatic effect may be difficult to avoid. Moreover, the choice of sites is limited.

In the present studies, cerebral blood flow was measured using the quantitative autoradiographic technique described by Sakurada et al. (1978). The benefits of this method are that it allows blood flow measurement in multiple, anatomically discrete regions of the brain in any animal. One limitation is that measurements can only be made once per animal. A major advantage of the ^{14}C-iodoantipyrine technique (Sakurada et al. 1978) is that it complements the autoradiographic technique for measuring the local cerebral glucose utilisation (Sokoloff et al. 1977), and this provides the technical capacity for rigorous investigations of the relationship between blood supply and metabolic demand.

The inter-regional differences in the cerebrovascular response to nimodipine administration have not been studied in

detail previously. Haws et al. (1983) examined, with radioactive microspheres, the effects of nimodipine in five gross anatomic regions of rabbit brain (cortex, caudate, brain stem, cerebellum and white matter) and observed proportionately similar elevations in each region after nimodipine administration. In the present study, using [^{14}C]-iodoantipyrine autoradiography to assess CBF at a large number of well-defined neuroanatomical locations, there was a clear regional heterogeneity in the alteration in local CBF provoked by nimodipine administration.

There were clear rostro-caudal differences in the cerebrovascular effects of nimodipine, with marked increases in CBF rostrally and minimal changes caudally. Thus, the most marked increases in CBF (by more than 60%) were observed in the rostral neocortex (parietal, auditory, sensory-motor and frontal cortices); moderate increases (25-50%) were observed in various subcortical forebrain regions (caudate nucleus, globus pallidus, hypothalamus and some thalamic nuclei) and some components of the limbic system (hippocampus, septal nuclei and amygdala). In sharp contrast, blood flow in the cerebellum, lower brain stem and white matter was minimally altered by the infusion of nimodipine (Table 19). The difference between these findings and the rather homogeneous elevations of CBF described by Haws et al. (1983) may reflect better spatial resolution provided by [^{14}C]-iodoantipyrine as compared to microspheres.

In addition, the same group demonstrated no change in CBF following nimodipine administration, and showed that the drug inhibits autoregulation of cerebral blood flow in response to hypertension in anaesthetised cats or monkeys (Haws and Heistad, 1984). This is in contrast to previous studies (Harris et al 1982; Symon et al. 1982; McCalden et al. 1984) in which nimodipine

was found to have little effect on autoregulation during change in arterial blood pressure in baboons. The reason for the reported differences in direct effects of nimodipine on CBF in the two studies from the same group (Haws et al. 1983; Haws and Heistad, 1984) is not clear; they used the same method of measurement of blood flow (radiolabelled microsphere) and the same dose of nimodipine. This discrepancy in their data may be attributed to difference in species (rabbit: Haws et al. 1983; cats and monkeys: Haws and Heistad, 1984). It is more likely that it is explained by the differences in experimental design. In their later studies in cats and monkeys, half of the control measurements of CBF were obtained 1-2 hours after stopping nimodipine infusion. It should be noted that the biological half-life ($t_{\frac{1}{2}}$) of nimodipine is relatively short, and is reported to be about 10 min in the baboon (Harris et al. 1982).

1.2 The effects of nimodipine on LCBF and LCGU in conscious animals.

In normal brain there is a close association, globally and locally, between the level of CBF and the level of energy generation. The latter is almost exclusively derived from the oxidative catabolism of glucose (Edvinsson and MacKenzie, 1977; Kuschinsky and Wahl, 1978; Siesjö, 1978; Kuschinsky, 1982-1983). It is increasingly recognised that effects on cerebral tissue metabolic activity are often the primary mechanism underlying the alterations in CBF that result from the administration of neurotransmitters or drugs. Although the direct actions of

calcium antagonists on the cerebral vasculature have been investigated extensively (Allen and Bahr, 1979; Allen and Banghart, 1979; Edvinsson et al. 1979; White et al. 1982; Andersson et al. 1983; Auer et al. 1983; Brandt et al. 1983), there have been no complete systematic investigations of cerebral glucose utilisation.

It has been shown that nimodipine increased hemispheric blood flow significantly, but had no effect on cerebral oxygen uptake and no obvious increase in electrocortical activity (Harper et al. 1981). These observations have been confirmed by several studies (Kazda et al. 1982; Haws et al. 1983; Steen et al. 1983; McCalden et al. 1984). Two lines of evidence indicate that calcium antagonists may have actions on cerebral tissue distinct from their effects on cerebral vessels. Firstly, several workers have demonstrated the presence of high-affinity binding sites for nimodipine and other dihydropyridine calcium antagonists in CNS as well as other tissues (cardiac, skeletal and smooth muscle), and there is good evidence to believe that these highly specific binding sites, at least in peripheral tissue, represent the locus of action of calcium antagonists (Bellemann et al. 1982; Bolger et al. 1982; Ehlert et al. 1982; Fairhurst et al. 1983; Murphy and Snyder, 1982; Peroutka and Allen, 1983; Luchowski et al. 1984). Autoradiographic studies with $[^3H]$-dihydropyridine agents suggest that the density of binding in synaptic areas of the brain is much greater than in blood vessels (Quirion, 1983; Ferry et al. 1984). Secondly, the administration of calcium antagonists can produce overt, though subtle, alterations in behaviour (Hoffmeister et al. 1982; Shah et al. 1983), and

changes in cerebral fucntion are generally reflected in local alterations in cerebral metabolic activity (Sokoloff, 198la,b, 1982; McCulloch, 1982).

In the present study, the relationship between local CBF and local cerebral glucose utilisation was studied at only one dose level of nimodipine (1 $\mu g\ kg^{-1} min^{-1}$), as this dose has been shown to have nearly maximal effects on local CBF with minimal effects on arterial blood pressure (McCalden et al. 1984; Mohamed et al. 1984) and plasma glucose concentration (Mohamed et al. 1984). The infusion of nimodipine at this concentration produced only a small (8%) reduction in the MABP which failed to reach statistical significance. The administration of nimodipine did not alter significantly the rate of glucose utilisation in any of the regions examined. By contrast, in 24 regions CBF was increased significantly by 39-84% from control level (for example, cerebral cortices, hippocampus, hypothalamus and most thalamic nuclei). Bellemann et al. (1983) identified the high affinity of binding receptors for nimodipine. They reported that the cerebral cortex and hippocampus are rich in binding sites for nimodipine, whereas the region of the pons is a poor source of radiolabelled calcium antagonists. The present data showed the LCBF in cerebral cortices increased significantly, 84% in parietal cortex and 64% in auditory cortex, whereas the LCBF in pons increased by 12% but did not reach statistically significant level. In vehicle treated animals there was an excellent correlation ($p < 0.01$) between the local levels of CBF and glucose utilisation where the ratio of flow to glucose use was approximately 1.5 ml μmol^{-1} in each brain region. During nimodipine treatment there was a similarly excellent correlation ($p < 0.01$) between

LCBF and LCGU, but the median ratio between local flow and glucose use increased to 2.5 ml μmol^{-1}.

As shown in the present investigation, nimodipine at an infusion concentration with marked cerebrovascular effects did not alter the rate of glucose utilisation in any CNS region. In view of the proven utility of the $[^{14}C]$-2-deoxyglucose technique to map functional events in the CNS as they are reflected in local rates of glucose utilisation (McCulloch, 1982; Sokoloff, 1982), the findings provide no evidence that nimodipine has other than minimal effects on functional activity in cerebral tissue, at least under the conditions of the present study. However, under the same conditions, nimodipine effected marked increases in CBF in most CNS areas, and the magnitude of the local flow changes observed is in general accord with those observed with previous, more global measurements (Harper et al. 1981; Kazda et al. 1982; Haws et al. 1983; McCalden et al. 1984).

In sharp and puzzling contrast to the findings of the present study (see also Mohamed et al. 1983, 1985) is the report by D'Avella et al. (1984) that nimodipine at a dose of 2 $\mu g\ kg^{-1}$ increased the LCGU (measured by the $[^{14}C]$-2-deoxyglucose technique) in twenty anatomically discrete regions of the CNS. These areas included the cerebellar hemisphere (by 191%), cerebellar nuclei (by 104%), visual cortex (by 118%), cerebellar white matter (by 96%) and corpus callosum (by 99%). There were no changes in the behaviour of the animals, even although these changes in LCGU are as great as those found in seizure activity by other authors (Collins, 1978; Caveness et al. 1980; Ingvar and Shapiro, 1981; Ingvar and Siesjö, 1983). We have

repeated in our laboratory the experimental protocol of D'Avella et al. (1984), and nimodipine failed to find any increases in the level of LCGU in any region of the CNS after a bolus of nimodipine (2 $\mu g\ kg^{-1}$).

Under normal conditions, an excellent correlation exists between the local levels of CBF and the local rates of glucose utilisation in all regions throughout the CNS (Sokoloff, 1981b,c). In many pharmacological and pathological states, the relationship between local flow and glucose use is maintained, and the ratio of blood flow to glucose utilisation remains unaltered in all areas. Alternatively, this can be demonstrated as no change in the intercept, α, of the log - log glucose use - CBF plots (Figure 20), or as no change in the slope derived from linear regression analysis of glucose utilisation (Figure 18). Under these conditions of unaltered flow-metabolism coupling, observed changes in CBF are usually attributed to concomitant alterations in underlying cerebral metabolic activity, implicating cerebral tissue and not cerebrovascular smooth muscle as the primary site of drug action. The type of response where the flow - glucose use relationship is undisturbed has been observed previously with barbiturates (Sokoloff, 1981a), the dopamine receptor agonist, apomorphine (McCulloch et al. 1982), and the γ-aminobutyric acid receptor agonist, muscimol (Kelly and McCulloch, 1983a). In the second pattern of response, either the relationship between flow and glucose use is significantly disturbed highly focally in a restricted number of regions in pathological conditions, such as in the vicinity of brain tumours (Blasberg et al. 1981; Mies et al. 1981), in cerebral ischaemia (Pulsinelli et al.1982b), or following administration of convulsants (Celik et al. 1982; Ingvar and Siesjö, 1983); or the disturbance of

the flow - glucose use relationship is widespread throughout the CNS. In these cases there is often an excellent correlation between flow and glucose use, but a marked alteration, in almost every region, in the magnitude of the ratio of blood flow to glucose use. This type of response has been noted previously with indomethacin, where the ratio of flow to glucose use is reduced throughout the brain (Pickard, 1981), with chronic metabolic acidosis, with noradrenaline-induced hypertension, and with γ-hydroxybutyrate administration, where the flow to glucose use ratio throughout the brain is increased (Kuschinsky et al. 1981, 1982, 1983, 1985). The changes in the flow - glucose use relationship that resulted from nimodipine administration are of this latter type, and the rather homogeneous alteration of this relationship throughout the CNS implicates a nimodipine-sensitive process (almost certainly calcium influx into cerebrovascular smooth muscle) (Bolton, 1979; Cauvin et al. 1982, 1983; Kawaguchi et al. 1982; Pan and Janis, 1984) as an integral component of the normal mechanisms via which blood flow is adjusted in vivo.

Evidence that nimodipine is beneficial in the treatment or prevention of cerebrovascular diseases is at present not conclusive. There are clinical reports that nimodipine may be of benefit in the prevention of vasospasm following a subarachnoid haemorrhage and, in these circumstances, may elevate CBF in patients (Brawanski et al. 1982; Gaab et al. 1982; Allen et al. 1983; Gelmers, 1982, 1984). Furthermore, Auer et al. (1983) have shown that nimodipine may dilate cerebral blood vessels in patients during extracranial-intracranial bypass surgery. What has not been established is whether these drugs will be more effective in the prophylaxis than in

the treatment of established brain damage. The results of the first part of the present studies clearly emphasise that, whatever the disease, the dose must be regulated very carefully to avoid hypotension and hyperglycaemia; with calcium antagonists, correct dose selection may therefore be critical. In addition, the observation that the increases in CBF produced by nimodipine are not associated with any potentially damaging increases in cerebral metabolism may be an important element in the use of drugs such as nimodipine in the treatment of cerebrovascular disease. The aim of the second part of this thesis was to determine the effects of nimodipine in a well defined experimental ischaemic insult.

2. The Effects of Nimodipine on a Focal Ischaemic Lesion.

2.1 The effect of nimodipine pre-treatment on LCBF following MCA occlusion.

One common cause of stroke in man is the occlusion of a brain vessel, usually arterial, or the spontaneous rupture of an intracranial artery with consequent haemorrhage in the brain parenchyma or in the subarachnoid space (Walker and Marx, 1981). A major consideration in the evaluation of experimental models of ischaemic stroke in animals is the clinical relevance of the models. Waltz (1979) has suggested that the proximal MCA occlusion is probably equivalent to a large cerebral infarct in man.

A wide variety of experimental models of cerebral ischaemia are available: global cerebral ischaemia has been performed by asphyxiation (Meyer, 1958; Cantu et al. 1969; Ames, 1975; Reichman, 1976); hemispheric ischaemia can be produced by ligation of the internal carotid artery or by injection of large emboli via the carotid artery (Fisher, 1951; Brown and Brierley, 1973; Molinari et al. 1974; Levinthal et al. 1979); multifocal cerebral ischaemia can be induced either by microspheres or autologus blood clot injection (Penry and Netsky, 1960; Rumbaugh et al. 1968; Bremer et al. 1975); focal cerebral ischaemia has been performed by occlusion of the middle cerebral artery in various experimental animals, such as primates (Crowell et al. 1970; Hudgins and Garcia, 1970; Ginsberg and Myers, 1972; Garcia, 1973; Aoyagi et al. 1975; Kawamura et al. 1975; Symon et al. 1979), cats (O'Brien and Waltz, 1973; Hayakawa and Waltz,

1975; Heiss et al. 1976; Katzman et al. 1977; Tamura et al. 1979), and dogs (Molinari and Laurent, 1976).

More recently, Tamura et al. (1981a) and Tyson et al. (1984) have described a technique for occluding the MCA in rats. The rat model has many advantages. There are close similarities between the anatomy of the cranial circulation of the rat and man (Yamori et al. 1976), and there is considerable information about various neurotransmitter systems, neurochemistry, neuroanatomy and neuropharmacology of the rat brain (Iversen et al. 1978; Roberts et al. 1978; Siesjö, 1978; Brown and Cooper, 1979). Also, it is in the rat, as a result of technical innovations, that a number of reliable quantitative techniques have become available that allow the regional assessment of cerebral blood flow (Sakurada et al. 1978), the permeability of the blood-brain barrier (Blasberg et al. 1978; Ohno et al. 1978), and the rates of cerebral glucose utilisation (Sokoloff et al. 1977) and protein synthesis (Smith et al. 1980).

The production of ischaemic damage by MCA occlusion (Tamura et al. 1981a) does not depend on combining arterial occlusion with hypoxia (Levine, 1960; Brown and Brierley, 1968; Salford et al. 1973), hypotension (Eklöf and Siesjö, 1972; Nordstrom and Rehncrona, 1977) or upon anatomical variations of the circle of Willis (Levine and Payan, 1966; Levy et al. 1975). Furthermore, in this study the use of quantification methods for measuring the amount of ischaemic damage has been shown to be reproducible (Graham et al. 1984) and to have advantages over the techniques such as grid planimetry (Sundt and Waltz, 1967; Michenfelder et al. 1976). However, the occlusion of an intracranial artery involves opening of the skull and subarachnoid space; thus, it could produce local brain damage.

The potency of calcium antagonists in preventing and reversing the contraction of cerebrovascular smooth muscle has resulted in great interest in the potential use of this group of compounds in the treatment of cerebrovascular disease.

Calcium antagonists have a variety of physiological effects that, on theoretical bases, may prove therapeutically effective in ischaemic brain. These include enhancement of CBF (Harper et al. 1981; Symon et al. 1982; Haws et al. 1983), selective dilatation of cerebrovascular vessels (Allen and Bahr, 1979; Allen and Banghart, 1979; Shimizu et al. 1980; Brandt et al. 1983; Takagi et al. 1983) and inhibition of vascular contraction due to substances such as serotonin (Brandt et al. 1981a,b; Kazda and Towart, 1981; Towart, 1981).

The effect of nimodipine on blood glucose level may also be important. Myers (1979) showed that the recovery following ischaemia was influenced by the plasma glucose level. it was observed that if animals were fed or infused with glucose prior to induction of ischaemia, recovery was adversely affected. These observations have been confirmed by several groups (Siemkowicz and Hansen, 1978; Ginsberg et al. 1980; Rehncrona et al. 1980, 1981; Welsh et al. 1980; Kalimo et al. 1981). Thus, there is experimental evidence that hyperglycaemia may be harmful in cerebral ischaemia (Gardiner et al. 1982; Pulsinelli et al. 1982a). In the first part of the present study a dose-dependent increase in plasma glucose level was observed during infusion of nimodipine (increased by 44 $\pm$ 2% after 30 min of nimodipine, 4 $\mu g\ kg^{-1} min^{-1}$). The elevated plasma glucose concentration during nimodipine therapy, first noted in the present study, may counteract any beneficial vascular effects either in animal models of cerebral ischaemia

or in patients with cerebrovascular disease.

In the investigations involving nimodipine pre-treated animals, the flow values in the hemisphere contralateral to MCA occlusion were greater than those in corresponding sites in vehicle treated rats by a factor very similar to that observed in normal rats. This indicates that the experimental lesion did not impair general cerebrovascular reactivity. In the hemisphere ipsilateral to the MCA occlusion, the control rats showed an area of profound ischaemia which conformed to the pattern previously described in this model (Tamura et al. 1981a,b; Tyson et al. 1984).

Inspection of the autoradiograms and analysis of the measurements of local cerebral blood flow showed that nimodipine pre-treatment significantly lessened the fall in local CBF in the peripheral part of the ischaemic area of the cortex. Thus, in visual cortex, a region supplied by both middle and posterior cerebral arteries, LCBF in the occluded hemisphere of control rats fell to 40% of that in the contralateral hemisphere, but in nimodipine pre-treated rats the LCBF remained at 91% of the value in the contralateral hemisphere in control rats. Even in sensory-motor cortex, the area of deepest cortical ischaemia, local CBF in nimodipine treated rats was twice that of vehicle treated rats (61 $\pm$ 9 versus 30 $\pm$ 3 ml $100g^{-1}min^{-1}$). By contrast, nimodipine preloading did not influence local CBF in the neostriatum, the area of maximum ischaemia. This may relate to anatomical blood supply consideration; middle cerebral artery originates from the circle of Willis near the junction with the internal carotid artery. A few millimetres distally, it gives off several ascending branches to the basal ganglia (lenticulostriatal arteries). Since the branches to the basal ganglia

are end-arteries, collateral blood supply is small, particularly when the occlusion is proximal to the point of branching, i.e., the occlusion includes the lenticulo-striate artery. One would expect, therefore, the lack of effect of nimodipine upon blood flow in the neostriatum is presumably because of the poor collateral supply to this region. Here, compared to local CBF in the contralateral hemisphere of control rats, flow was reduced by 93% in both treated and control groups. Nevertheless, there were no areas of the brain in treated rats that showed values lower than control rats. Despite the powerful vasodilatory effects of nimodipine, it did not cause an intracerebral steal effect.

In the introduction (Chapter I, Section 8) it was pointed out that a vasodilator could have several effects in the presence of a focal ischaemic lesion. These include: 1) generalised increase in CBF both in ischaemic and "normal" areas; 2) a deleterious redistribution, or steal; and 3) a beneficial redistribution. A consideration of the frequency distributions of blood flow suggests that it is the first of these three that best explains the effects of nimodipine pre-treatment observed in this study.

The frequency distribution of the level of CBF was measured bilaterally (ipsilateral and contralateral to MCA occlusion in animals receiving vehicle or nimodipine) in the total area of the hemisphere at the level of the sensory-motor cortex. Nimodipine had similar effects upon the ischaemic area and the total hemispheric blood flows (Figures 26 and 27). For example, in animals receiving vehicle, the total hemispheric area with blood flow less than or equal to 25 ml $100g^{-1}min^{-1}$ was 8 $\pm$ 2% (contralateral) and 46 $\pm$ 3 (ipsilateral), and in nimodipine pre-treated

animals the area of hemisphere with blood flow less than or equal to 25 ml $100g^{-1}min^{-1}$ was 5 ± 1% (contralateral) and 29 ± 6% (ipsilateral); therefore, in both vehicle and nimodipine treated animals the area with CBF less than or equal to 25 ml $100g^{-1}min^{-1}$ in ipsilateral hemisphere was some six-fold greater than that in the contralateral hemisphere. Furthermore, the median hemispheric blood flow in animals receiving vehicle was 28 ml $100g^{-1}min^{-1}$ (ipsilateral) and 105 ml $100g^{-1}min^{-1}$ (contralateral), and in nimodipine pre-treated groups was 46 ml $100g^{-1}min^{-1}$ (ipsilateral) and 169 ml $100g^{-1}min^{-1}$ (contralateral). The ratio of median values of CBF in the ipsilateral and contralateral hemispheres is, therefore, 73% in both vehicle and nimodipine treated animals.

The pattern of local CBF in focal ischaemia is determined by several factors (Tyson et al. 1984). It is not possible from the present study to identify precisely the reason for the effects of nimodipine on this pattern. The beneficial effect upon flow in the periphery of the ischaemic cortex implies that nimodipine pre-treated animals were better provided with collateral supply to those areas. It is not clear why pre-treated animals had a more efficient collateral input than that evoked by the ischaemic insult alone, although it is tempting to invoke an action of nimodipine in preventing the vasoconstriction that occurs in focal ischaemia (Teasdale et al. 1983). This would need to be confirmed by direct observation. Indeed, reversal of such vasoconstriction, which occurs only in the area of maximum ischaemia, might be expected to provoke a steal effect. The absence of steal effect in this study may indicate that this occurs only when vasodilator is administered after the initial haemodynamic effects of local occlusion have been established.

Although the beneficial effects of pre-treatment with nimodipine may result from several factors, it is most likely due to better oxygen delivery to areas of peripheral ischaemia as a consequence of the relative preservation of blood flow. The present results do not point to there having been an attenuation of ischaemic damage by modification of more subtle biochemical events, such as by the prevention of the entry of calcium into damaged neuron, but neither do they disprove it.

2.2 The effect of nimodipine post-treatment on LCBF following MCA occlusion.

The results of the commencement of nimodipine (1 $\mu g\ kg^{-1}\ min^{-1}$) <u>after</u> the production of a focal ischaemic lesion showed that the drug did not have beneficial effects on CBF. These findings, therefore, are in sharp contrast with the effect of nimodipine (1 or 2 $\mu g\ kg^{-1} min^{-1}$) infused <u>before</u> middle cerebral artery occlusion.

The effect of the drug may be completely different depending on whether it is given before or after ischaemia, because different pathomechanisms of cell damage might be operating at different stages of ischaemia, and because changes in peripheral vascular tone and reactivity can occur after obstruction of a major cerebral artery (Waltz and Sundt, 1967; Symon et al. 1971; Symon et al. 1976; Teasdale et al. 1983).

The drug was infused for a total of 60 minutes before the CBF measurement in pre-treated animals, but only for 30 minutes in those post-treated groups. This may have led to different plasma concentrations of nimodipine at the time of the CBF measurement in the two groups but, in practice, the difference

is unlikely to be great. From the pharmacokinetic model (see Figure 2), it can be seen that the arterial plasma concentration of nimodipine (1 $\mu g\ kg^{-1} min^{-1}$) reaches its plateau level after 133 minutes of continuous infusion. After 30 and 60 minutes of infusion, the arterial plasma concentrations of nimodipine are calculated to be 65% and 88% of the plateau level, respectively. The lower levels of systemic arterial blood pressure noted at the time of CBF measurements in post-treated animals (see Table 31) suggest that the circulating concentrations of the drug were sufficient to dilate peripheral vessels (Tanaka et al. 1980; Auer et al. 1982), which are generally less sensitive to calcium antagonists (Towart and Kazda, 1980; Brandt et al. 198la). Moreover, the low level of local blood flow, with its slow delivery of the drug to the ischaemic area, may also be a limitation of post-treatment as compared to effective preloading of the brain with nimodipine prior to the ischaemic insult.

Another factor which may limit the effects of post-treatment is the profound alterations in the reactivity of cerebral vessels that occur during ischaemia. Indeed, as well as impairment of CO_2 reactivity (Hossmann et al. 1973; Symon et al. 1974) after MCA occlusion, the vasodilatory response of pial arterioles to locally applied calcium antagonists is reduced (Brandt et al. 1983). it is, therefore, quite possible that the cerebrovascular response to systemically administered nimodipine differs according to whether it is given before or after occlusion.

The same factors that may have limited the effect of the drug on the ischaemic hemisphere when given after occlusion may also have been responsible for the finding that blood flow was

not increased in the opposite hemisphere. However, another mechanism that might have reduced cerebrovascular reactivity in that hemisphere is the phenomenon of so-called diaschisis (Monakow, 1941). Tanaka et al. (1980) have shown that CBF is indeed reduced in areas remote from the ischaemic lesion. These effects would have been more extensive in post-treated animals than in the pre-treated series. In any event, the present findings raise the possibility that nimodipine may be "protective", but not that it is "resuscitative".

2.3 The effect of nimodipine pre-treatment on the pathological consequences.

In this model, flow levels of less than 30 ml $100g^{-1}min^{-1}$ are associated with the development of ischaemic damage (Tamura et al. 1981b; Tyson et al. 1984). In nimodipine pre-treated animals CBF was preserved above this level in parts of the cerebral cortex (the peripheral and penumbral areas of the lesion); this is consistent with the neuropathological observation that none of the nimodipine pre-treated animals had ischaemic lesion as large as seen in some of the control rats. On the other hand, the lack of effect of the drug upon profoundly ischaemic tissue in the neostriatum explains the observation that the ischaemic damage was not ameliorated in all animals.

One major finding of this study is that the effects of nimodipine are less pronounced when assessed by volumetric analysis of ischaemic damage than when assessed as individual levels of CBF at corresponding sites within the lesion. Various technical and biological explanations for the lack

of precise matching of the volume of ischaemic flow thresholds are possible: these include the necessity for performing the two studies in separate parallel groups with dissimilar time constants, the single time point of the CBF measurements relative to the drug dosage over the first hour and the possibility that CBF thresholds for ischaemic damage might vary in different brain regions.

Previous studies on the effects of various calcium antagonists on cerebral ischaemic lesion are summarised in Table 33. It can readily be seen that these studies have employed different species, different lesions, different drugs and different schedules of administration. Moreover, different methods have been used to measure CBF, and also to assess the severity of the ischaemic brain damage. It is therefore not surprising that the results are conflicting! The calcium antagonist, flunarizine, has been reported to be a potent inhibitor of calcium-induced constriction of isolated arteries, being most effective in cerebral artery (van Neuten and Vanhoutte, 1981). Recently, White et al. (1982) reported that flunarizine not only improved but even increased cerebral cortical blood flow above pre-ischaemic level for 90 minutes following a 20 minute period of circulatory arrest in dog maintained on cardio-pulmonary bypass. By contrast, Newberg et al. (1984) demonstrated that flunarizine failed to improve the post-ischaemic CBF and the neurological outcome, this in agreement with Hossmann et al. (1983) who reported the failure of this drug to prevent post-ischaemic accumulation of calcium or to improve cortical electrical function or biochemical recovery.

The studies that are most relevant to the present experiments were reported by Harris et al. (1982) and Reedy et al. (1983).

In both, the ischaemic lesion was produced by occlusion of the middle cerebral artery, and also in both, the animals were pre-treated with calcium antagonist. Harris et al. (1982), like the present investigation, used nimodipine and observed that CBF was higher in treated animals, but reported that oedema was increased and calcium/potassium homeostasis was disturbed in nimodipine treated animals. They therefore doubted that nimodipine pre-treatment would result in overall benefits. If nimodipine enhances tissue perfusion in regions which are irreversibly damaged, then these cerebral circulatory changes may not be of any therapeutic benefit because of the risks of oedema (Harris et al. 1982). However, it is important to note that Harris et al. (1982) utilised hypotension in addition to occlusion in order to produce their lesion; this additional insult would have produced other systemic effects, such as hyperglycaemia and elevation of circulating catecholamines, so these would be likely to exacerbate the tissue effects of ischaemia (Siemkowicz and hansen, 1978; Pulsinelli et al. 1982a). In the present study, conditions were carefully controlled and there were minimal variations in arterial blood pressure (a maximum reduction of 8% with nimodipine 1 $\mu g\ kg^{-1}\ min^{-1}$ pre-treatment) or in $PaCO_2$, PaO_2 or arterial blood glucose.

Reedy et al. (1983) were unable to observe an effect upon CBF in animals treated with a calcium antagonist. They used verapamil, a weaker agent (Towart et al. 1982; Andersson et al. 1983), in a dose that may have been inadequate.

Beneficial effects on ischaemic brain damage, as judged either by neurological outcome, survival, or electrophysiological parameters, have been reported after either pre-treatment with nimodipine (Hoffmeister et al. 1979; Steen et al. 1983)

or post-treatment (Gelmers, 1984; Steen et al. 1984). Smith et al. (1983) have reported on the lack of benefit from nimodipine in terms of the EEG after pre-treatment of global ischaemia. Other reports of lack of benefit of calcium antagonists upon tissue calcium content, and upon the neuropathological signs of ischaemic damage (Hossmann et al. 1983; Reedy et al. 1983), concern either verapamil (Reedy et al. 1983), in a dose that did not affect CBF, flunarizine post-treatment (Hossmann et al. 1983; Newberg et al. 1984) or lidoflazine (Dean et al. 1984).

Despite the differences in ischaemic model, agent, and method of administration, the most critical factor in determining the effects of a calcium antagonist upon an ischaemic insult may be the stage that treatment is instituted. The study provides convincing experimental evidence that pre-treatment with nimodipine could have beneficial effects in improving blood flow and in limiting cerebral damage in areas of brain adjacent to the "core" of a focal ischaemic lesion. However, nimodipine failed to modify the CBF pattern when given after the ischaemic insult. Pre-treatment with a calcium antagonist, therefore, may be most useful clinically in patients identified to be at risk of developing focal ischaemia, rather than in the treatment of established lesions.

There is already some clinical evidence that prophylactic treatment with nimodipine may be beneficial in prevention of ischaemia deficits due to "vasospasm" resulting from subarachnoid haemorrhage (Allen et al. 1983). The findings of the present study are therefore of considerable relevance to patients with subarachnoid haemorrhage and patients with warning symptoms of occlusive cerebrovascular disease.

TABLE 33. EXPERIMENTAL AND CLINICAL STUDIES WITH CALCIUM ANTAGONISTS.

EXPERIMENTAL STUDIES

Nimodipine pre-treatment.

Reference	Species	Schedule of Administration	Anaesthesia	Ischaemic Insult	CBF Method	CBF Effect	Other Effects
Harris et al. 1982	Baboon	0.6 $\mu g\ kg^{-1}min^{-1}$ I.A.	α-Chloralose	Focal + hypotension	Hydrogen clearance	25% > control	Increased oedema
Kazda et al. 1982	Cat	1 $mg\ kg^{-1}$ oral	Ketamine	Complete global	^{133}Xe	20% > control	-
Steen et al. 1983	Dog	1 $\mu g\ kg^{-1}min^{-1}$ I.V.	Halothane	Complete global	Venous outflow	Two-fold increase	Outcome improved
Smith et al. 1983	Rat	1 $mg\ kg^{-1}min^{-1}$ I.V.	Halothane	Forebrain	^{14}C-IAP	Increase in many regions	No effect on EEG
Hoffmeister et al. 1979	Rat Mouse Cat	1 $mg\ kg^{-1}$ oral	-	Complete global	-	-	Prolonged survival and improved EEG

Nimodipine post-treatment.

Steen et al. 1984	Dog	1 $\mu g\ kg^{-1}min^{-1}$ I.V.	Halothane	Complete global	Venous outflow	Two-fold increase	Outcome improved

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TABLE 33 (Contd.)

Verapamil pre-treatment.

Reference	Species	Schedule of Administration	Anaesthesia	Ischaemic Insult	CBF Method	CBF Effect	Other Effects
Reedy et al. 1983	Cat	0.2 mg $kg^{-1}min^{-1}$ I.V.	N_2O	Focal	^{133}Xe	None	No change in ischaemic damage.
Flunarizine post-treatment.							
Hossmann et al. 1983	Cat	0.1 mg kg^{-1} I.V.	Halothane	Complete global	-	-	No change in tissue calcium content
Newberg et al. 1984	Dog	6 µg kg^{-1} I.V.	Halothane	Complete global	Venous outflow	None	No change in neurologic recovery
Lidoflazine post-treatment.							
Dean et al. 1984	Dog	1 mg kg^{-1} I.V.	Sodium pentobarbital	Complete global	Radio-labelled microsphere	None	No change in neurologic recovery

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TABLE 33 (Contd.)

CLINICAL STUDIES

Nimodipine pre-treatment.

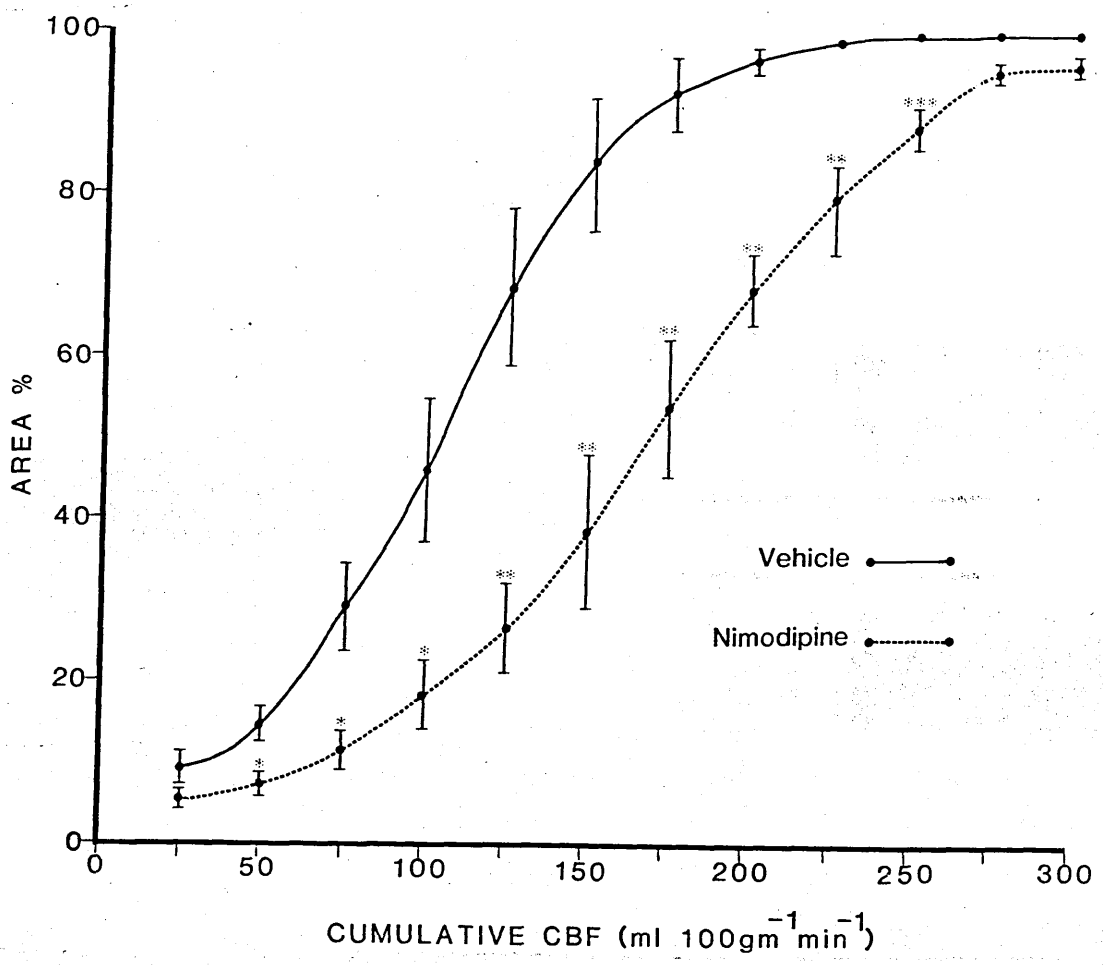
Reference	Species	Schedule of Administration	Anaesthesia	Ischaemic Insult	CBF Method	CBF Effect	Other Effects
Allen et al. 1983		0.35 mg kg^{-1} 4 hr^{-1} orally for 21 days	-	SAH	-	-	Reduced deficit

Nimodipine post-treatment.

Gaab et al. 1982		54 mg (mean dose) orally	-	SAH	Xenon inhalation	14% increase	-
Gelmers 1984		120 mg (3 times day^{-1}, orally)	-	Ischaemic stroke	-	-	Reduced deficit Increased c sciousness

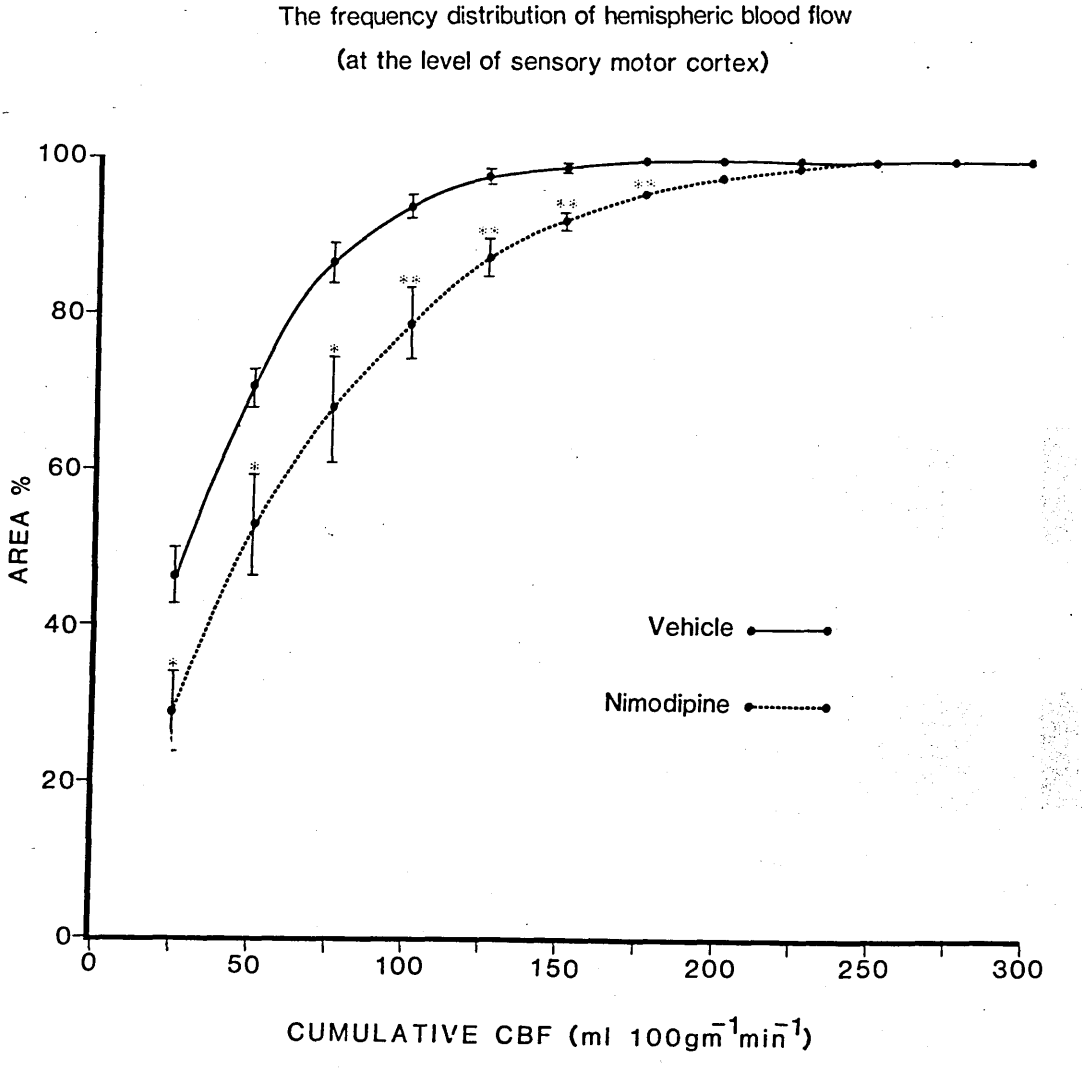
Figure 26.

The frequency distribution of hemispheric blood flow
(at the level of sensory motor cortex)



Data are presented as mean area (%) ± SE, with cumulative CBF values within each flow bin: 0-25, 0-50, 0-75, ... 0-300 ml $100g^{-1}min^{-1}$ for hemispheric blood flow; contralateral (vehicle, n-5) compared with contralateral (nimodipine, n=5).
*$p<0.05$; **$p<0.01$ and ***$p<0.001$. Student's unpaired t-test.

Figure 27.



Data are presented as mean area (%) ± SE, with cumulative CBF values within each flow bin: 0-25, 0-50, 0-75 .... 0-300 ml $100g^{-1}min^{-1}$ for hemispheric blood flow; ipsilateral (vehicle, n=5) compared with ipsilateral (nimodipine, n=5).
*$p < 0.05$; **$p < 0.01$ (Student's unpaired t-test).

APPENDICES

TABLE 1. ERRORS INVOLVING KILL-TIME (MODEL A, 30 SEC).

CBF (base line) T = 0.48	CBF T = 0.46	CBF T = 0.44	CBF T = 0.50	CBF T = 0.52
50	54	60	46	43
100	109	120	92	85
150	165	182	137	126
200	221	245	182	167
250	277	310	227	207
300	335	377	271	245
350	393	446	314	283
400	452	518	357	320

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of kill-time within a value of $\pm$ 2 seconds. (T = the time in deciminutes).

TABLE 2. ERRORS INVOLVING KILL-TIME (MODEL A, 60 SEC).

CBF (base line) T = 0.98	CBF T = 0.96	CBF T = 0.94	CBF T = 1.0	CBF T = 1.02
50	52	55	48	46
100	105	110	95	91
150	158	167	142	135
200	212	225	189	178
250	267	287	234	220
300	324	350	279	260
350	379	412	324	300
400	437	479	365	337

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of kill-time within a value of $\pm$ 2 seconds. (T = the time in deciminutes).

TABLE 3. ERRORS INVOLVING KILL-TIME (MODEL B, 30 SEC).

CBF (base line) T = 0.48	CBF T = 0.46	CBF T = 0.44	CBF T = 0.50	CBF T = 0.52
50	67	86	36	26
100	139	184	71	51
150	115	298	104	74
200	297	432	136	96
250	384	591	166	116
300	477	784	196	135
350	577	-	224	154
400	682	-	252	171

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of kill-time within a value of $\pm$ 2 seconds. (T = the time in deciminutes).

TABLE 4. ERRORS INVOLVING KILL-TIME (MODEL B, 60 SEC).

CBF (base line) T = 0.98	CBF T = 96	CBF T = 94	CBF T = 1.0	CBF T = 1.02
50	60	70	42	35
100	123	150	82	68
150	190	242	120	98
200	261	344	157	127
250	334	456	193	153
300	411	576	228	179
350	489	700	261	203
400	570	-	294	225

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of kill-time within a value of $\pm$ 2 seconds. (T = the time in deciminutes).

TABLE 5. ERRORS INVOLVING KILL-TIME (MODEL C, 30 SEC).

CBF (base line) T = 0.48	CBF T = 0.46	CBF T = 0.44	CBF T = 0.50	CBF T = 0.52
50	52	55	48	46
100	105	110	95	91
150	158	166	143	137
200	210	221	191	183
250	262	276	238	228
300	315	332	286	274
350	367	386	335	320
400	419	441	380	363

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of kill-time within a value of $\pm$ 2 seconds. (T = the time in deciminutes).

TABLE 6. ERRORS INVOLVING KILL-TIME (MODEL C, 60 SEC).

CBF (base line) T = 0.98	CBF T = 0.96	CBF T = 0.94	CBF T = 1.01	CBF T = 1.02
50	51	52	49	48
100	102	105	98	96
150	154	158	146	144
200	205	210	195	191
250	257	263	245	238
300	308	316	294	286
350	355	363	337	329
400	404	410	382	367

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of kill-time within a value of $\pm$ 2 seconds. (T = the time in deciminutes).

TABLE 7. LAG-TIME CORRECTION AND RELATIONSHIP TO THE CBF.

(MODEL A: 30 SEC).

CBF (base line) Lag-time 0.01	CBF Lag-time 0.02	CBF Lag-time 0.03	CBF Lag-time 0.04
50	48	46	45
100	96	92	89
150	144	138	133
200	191	183	175
250	238	228	218
300	285	272	259
350	332	315	300
400	377	359	339

Variability of CBF (ml $100g^{-1}min^{-1}$) related to lag-time correction within a value of + 0.01, + 0.02 or + 0.03.

TABLE 8. LAG-TIME CORRECTION AND RELATIONSHIP TO THE CBF.

(MODEL A: 60 SEC).

CBF (base line Lag-time 0.01	CBF Lag-time 0.02	CBF Lag-time 0.03	CBF Lag-time 0.04
50	49	48	47
100	98	96	94
150	146	143	139
200	194	189	184
250	242	234	228
300	290	280	270
350	337	324	312
400	382	366	351

Variability of CBF (ml $100g^{-1}min^{-1}$) related to lag-time correction within a value of + 0.01, + 0.02 or + 0.03.

TABLE 9. LAG-TIME CORRECTION AND RELATIONSHIP TO THE CBF.

(MODEL B: 30 SEC).

CBF (base line) Lag-time 0.01	CBF Lag-time 0.02	CBF Lag-time 0.03	CBF Lag-time 0.04
50	43	36	31
100	84	71	60
150	125	104	88
200	164	136	114
250	203	167	138
300	241	196	162
350	278	225	185
400	314	252	206

Variability of CBF (ml $100g^{-1}min^{-1}$) related to lag-time correction within a value of + 0.01, + 0.02 or + 0.03.

TABLE 10. LAG-TIME CORRECTION AND RELATIONSHIP TO THE CBF.

(MODEL B: 60 SEC).

CBF (base line) Lag-time 0.01	CBF Lag-time 0.02	CBF Lag-time 0.03	CBF Lag-time 0.04
50	46	42	39
100	90	82	75
150	134	120	109
200	177	157	141
250	219	193	172
300	260	228	201
350	301	261	229
400	341	294	257

Variability of CBF (ml $100g^{-1}min^{-1}$) related to lag-time correction within a value of + 0.01, + 0.02 or + 0.03.

TABLE 11. LAG-TIME CORRECTION AND RELATIONSHIP TO THE CBF.

(MODEL C: 30 SEC).

CBF (base line) Lag-time 0.01	CBF Lag-time 0.02	CBF Lag-time 0.03	CBF Lag-time 0.04
50	49	49	48
100	99	97	96
150	148	146	144
200	197	195	192
250	246	243	240
300	296	292	288
350	346	341	336
400	394	389	382

Variability of CBF (ml $100g^{-1}min^{-1}$) related to lag-time correction within a value of + 0.01, + 0.02 or + 0.03.

TABLE 12. LAG-TIME CORRECTION AND RELATIONSHIP TO THE CBF.
(MODEL C: 60 SEC).

CBF (base line) Lag-time 0.01	CBF Lag-time 0.02	CBF Lag-time 0.03	CBF Lag-time 0.04
50	50	50	49
100	99	99	98
150	139	148	147
200	198	197	195
250	249	247	245
300	299	297	294
350	344	341	336
400	391	385	380

Variability of CBF (ml $100g^{-1}min^{-1}$) related to lag-time correction within a value of + 0.01, + 0.02 or + 0.03.

TABLE 13. ERRORS INVOLVING THE PARTITION COEFFICIENT (LAMBDA). (MODEL A: 30 SEC).

CBF (base line) $\lambda = 0.8$	CBF $\lambda = 0.7$	CBF $\lambda = 0.6$	CBF $\lambda = 0.9$	CBF $\lambda = 1.0$
50	51	52	49	49
100	103	108	98	96
150	158	171	144	140
200	216	242	190	182
250	277	326	233	222
300	341	429	276	259
350	411	561	316	294
400	487	-	355	327

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of Lambda within a value of $\pm$ 0.1 or 0.02.

TABLE 14. ERRORS INVOLVING THE PARTITION COEFFICIENT (LAMBDA).

(MODEL A: 60 SEC).

CBF (base line) $\lambda = 0.8$	CBF $\lambda = 0.7$	CBF $\lambda = 0.6$	CBF $\lambda = 0.9$	CBF $\lambda = 1.0$
50	52	54	49	48
100	108	122	95	91
150	171	217	138	129
200	245	377	177	163
250	334	-	213	192
300	446	-	246	218
350	586	-	275	239
400	-	-	303	258

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of Lambda within a value of $\pm$ 0.1 or $\pm$ 0.02.

(- indicates the failure of computer to convert the new blood flow values).

TABLE 15. ERRORS INVOLVING THE PARTITION COEFFICIENT (LAMBDA).
(MODEL B: 30 SEC).

CBF (base line) $\lambda = 0.8$	CBF $\lambda = 0.7$	CBF $\lambda = 0.6$	CBF $\lambda = 0.9$	CBF $\lambda = 1.0$
50	50	51	50	49
100	102	104	99	98
150	154	160	147	144
200	207	218	194	190
250	261	278	242	235
300	316	339	288	279
350	371	403	335	323
400	427	468	380	366

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of Lambda within a value of $\pm$ 0.1 or $\pm$ 0.02.

TABLE 16. ERRORS INVOLVING THE PARTITION COEFFICIENT (LAMBDA). (MODEL B: 60 SEC).

CBF (base line) $\lambda = 0.8$	CBF $\lambda = 0.7$	CBF $\lambda = 0.6$	CBF $\lambda = 0.9$	CBF $\lambda = 1.0$
50	51	52	49	49
100	103	109	97	95
150	158	169	144	140
200	213	232	191	183
250	269	297	236	226
300	326	364	281	267
350	383	432	326	308
400	441	500	370	348

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of Lambda within a value of $\pm$ 0.01 or 0.02.

TABLE 17. ERRORS INVOLVING THE PARTITION COEFFICIENT (LAMBDA). (MODEL C: 30 SEC).

CBF (base line) $\lambda = 0.8$	CBF $\lambda = 0.7$	CBF $\lambda = 0.6$	CBF $\lambda = 0.9$	CBF $\lambda = 1.0$
50	51	53	49	49
100	105	113	96	94
150	163	187	142	136
200	227	293	184	174
250	303	550	224	208
300	399	-	260	238
350	548	-	293	264
400	-	-	322	285

Variability of CBF (ml $100g^{-1}min^{-1}$) related to underestimation or overestimation of Lambda within a value of $\pm$ 0.01 or $\pm$ 0.02.

(- indicates the failure of computer to convert the new blood flow values).

APPENDIX II

Errors associated with optical density measurements.

The blood samples data, including the time, the radioactivity concentration and the weight of each sample is stored in the computer, and the CBF programme is carried out. The optical densities which correspond to flows of 50, 100, 150 ... 400 are obtained. The corresponding optical density within a value of $\pm$ 10% to each blood flow level is calculated. These steps are performed for Models A, B and C when the isotope is infused over 30 or 60 second periods (see Tables 18-23).

TABLE 18. ERRORS INVOLVING THE OPTICAL DENSITY (OD) MEASUREMENTS AND RELATIONSHIP TO CBF. (MODEL A: 30 SEC).

OD Base line	CBF Base line	OD + 10%	CBF + 10%	OD - 10%	CBF - 10%
0.204	50	0.224	56	0.183	44
0.370	100	0.407	112	0.333	88
0.508	150	0.558	170	0.457	130
0.622	200	0.684	231	0.559	171
0.718	250	0.789	294	0.646	211
0.798	300	0.877	360	0.718	250
0.865	350	0.951	428	0.778	287
0.922	400	1.014	501	0.829	322

CBF (ml $100g^{-1}min^{-1}$) levels and relationship to the optical density measured by the Quantimet densitometer within a value of $\pm$ 10%.

TABLE 19. ERRORS INVOLVING THE OPTICAL DENSITY (OD) MEASUREMENTS AND RELATIONSHIP TO CBF. (MODEL A: 60 SEC).

OD Base line	CBF Base line	OD + 10%	CBF + 10%	OD - 10%	CBF - 10%
0.378	50	0.415	56	0.340	44
0.632	100	0.695	115	0.568	85
0.812	150	0.893	181	0.730	125
0.937	200	1.030	252	0.843	161
1.027	250	1.129	333	0.924	194
1.094	300	1.203	423	0.984	225
1.145	350	1.259	528	1.030	252
1.187	400	1.305	654	1.068	279

CBF (ml $100g^{-1}min^{-1}$) levels and relationship to the optical density measured by the Quantimet densitometer within a value of $\pm$ 10%.

TABLE 20. ERRORS INVOLVING THE OPTICAL DENSITY (OD) MEASUREMENTS AND RELATIONSHIP TO CBF. (MODEL B: 30 SEC).

OD Base line	CBF Base line	OD + 10%	CBF + 10%	OD - 10%	CBF - 10%
0.048	50	0.052	54	0.043	44
0.091	100	0.100	112	0.081	88
0.129	150	0.141	167	0.116	133
0.163	200	0.179	225	0.146	175
0.194	250	0.213	283	0.174	217
0.223	300	0.245	343	0.200	260
0.249	350	0.273	400	0.224	303
0.273	400	0.300	460	0.245	343

CBF (ml $100g^{-1}min^{-1}$) levels and relationship to the optical density measured by the Quantimet densitometer within a value of $\pm$ 10%.

TABLE 21. ERRORS INVOLVING THE OPTICAL DENSITY (OD) MEASUREMENTS AND RELATIONSHIP TO CBF. (MODEL B: 60 SEC).

OD Base line	CBF Base line	OD + 10%	CBF + 10%	OD - 10%	CBF - 10%
0.098	50	0.107	55	0.088	44
0.177	100	0.194	112	0.159	88
0.242	150	0.266	171	0.217	130
0.298	200	0.327	230	0.268	173
0.346	250	0.380	289	0.311	213
0.389	300	0.427	348	0.350	254
0.429	350	0.471	410	0.386	296
0.464	400	0.510	470	0.417	335

CBF (ml $100g^{-1}min^{-1}$) levels and relationship to the optical density measured by the Quantimet densitometer within a value of $\pm$ 10%.

TABLE 22. ERRORS INVOLVING THE OPTICAL DENSITY (OD) MEASUREMENTS AND RELATIONSHIP TO CBF. (MODEL C: 30 SEC).

OD Base line	CBF Base line	OD + 10%	CBF + 10%	OD - 10%	CBF - 10%
0.340	50	0.374	56	0.306	45
0.595	100	0.654	113	0.535	87
0.795	150	0.874	175	0.715	128
0.945	200	1.039	239	0.850	167
1.061	250	1.167	313	0.954	203
1.148	300	1.262	393	1.033	237
1.216	350	1.337	495	1.094	267
1.269	400	1.395	645	1.142	296

CBF (ml $100g^{-1}min^{-1}$) levels and relationship to the optical density measured by the Quantimet densitometer within a value of $\pm$ 10%

TABLE 23. ERRORS INVOLVING THE OPTICAL DENSITY (OD) MEASUREMENTS AND RELATIONSHIP TO CBF. (MODEL C: 60 SEC).

OD Base line	CBF Base line	OD + 10%	CBF + 10%	OD - 10%	CBF - 10%
0.625	50	0.687	60	0.562	42
0.950	100	1.045	119	0.855	83
1.157	150	1.272	198	1.041	118
1.275	200	1.402	333	1.147	147
1.342	250	1.476	-	1.207	168
1.383	300	1.521	-	1.244	185
1.410	350	1.551	-	1.269	197
1.425	400	1.567	-	1.282	204

CBF (ml $100g^{-1}min^{-1}$) levels and relationship to the optical density measured by the Quantimet densitometer within a value of $\pm$ 10%.
(- indicates the failure of computer to convert the new blood flow value).

APPENDIX III

Errors associated with underestimation of the tracer concentrations.

The blood samples data, sample time and the weight of each blood sample is stored in the computer. The CBF programme is run to give the corresponding concentration of each sample. The 1%, 5% and 10% concentration of each sample is calculated (for example see Table 24). By using another CBF programme the new blood flows, compared to those of the base line data, are calculated by knowing the time and the corresponding concentrations of the tracer for each sample. The CBF values for Models A, B and C within a value of -1%, -5% or -10% of underestimation of the tracer concentration are shown in Tables 24-30.

TABLE 24. BLOOD DATA AND SAMPLE TIME OF CBF.

(MODEL A: 30 SEC).

Sample	Time (Min)	Concentration Base line	Concentration -1%	Concentration -5%	Concentration -10%
1	0.00	0.0	0	0	0
2	0.07	283.8	280.9	269.6	255.4
3	0.09	378.4	374.6	359.4	340.5
4	0.12	473.0	468.2	449.3	425.7
5	0.17	662.2	655.5	629.1	595.9
6	0.19	756.8	749.2	718.9	681.1
7	0.22	851.4	842.8	808.8	766.2
8	0.24	945.9	936.4	898.6	851.3
9	0.27	1040.5	1030.1	988.4	936.4
10	0.29	1135.1	1123.7	1078.3	1021.6
11	0.32	1229.7	1217.4	1168.2	1106.7
12	0.34	1324.3	1311.1	1258.1	1191.8
13	0.37	1418.9	1404.7	1347.9	1277.0
14	0.39	1513.5	1498.3	1437.8	1362.1
15	0.42	1608.1	1591.9	1527.6	1447.2
16	0.44	1664.9	1648.2	1581.6	1498.4
17	0.47	1731.1	1713.7	1644.5	1557.9
Concentration (nCi/ml)		1770.8	1735.5	1665.5	1577.7

^{14}C-Iodoantipyrine blood sample concentration involved errors produced by the liquid scintillation analyser within a value of -1%, -5% or -10%. Kill-time = 0.48 min; catheter flow = 2.6 ml/min, and catheter lag-time = 0.01 min.

TABLE 25.

^{14}C-IODOANTIPYRINE UNDERESTIMATION AND RELATIONSHIP TO THE CBF (MODEL A: 30 SEC).

CBF Base line	CBF -1%	CBF -5%	CBF -10%
50	51	54	57
100	102	107	115
150	154	163	176
200	205	219	238
250	258	276	304
300	310	328	374
350	363	395	446
400	415	475	523

CBF (ml $100g^{-1}min^{-1}$) levels related to underestimation produced by liquid scintillation analyser within a value of -1%, -5% or -10% in the ^{14}C-iodoantipyrine concentration.

TABLE 26.

^{14}C-IODOANTIPYRINE UNDERESTIMATION AND RELATIONSHIP TO THE CBF (MODEL A: 60 SEC).

CBF Base line	CBF -1%	CBF -5%	CBF -10%
50	51	53	57
100	101	108	118
150	153	166	185
200	205	226	266
250	257	290	346
300	311	356	444
350	362	428	562
400	419	509	706

CBF (ml $100g^{-1}min^{-1}$) levels related to underestimation produced by liquid scintillation analyser within a value of -1%, -5% or -10% in the ^{14}C-iodoantipyrine concentration.

TABLE 27.

^{14}C-IODOANTIPYRINE UNDERESTIMATION AND RELATIONSHIP TO THE CBF (MODEL B: 30 SEC).

CBF Base line	CBF -1%	CBF -5%	CBF -10%
50	54	56	59
100	108	114	121
150	163	172	184
200	219	231	248
250	275	291	314
300	334	354	383
350	391	416	451
400	449	479	522

CBF (ml $100g^{-1}min^{-1}$) levels related to underestimation produced by liquid scintillation analyser within a value of -1%, -5% or -10% in the ^{14}C-iodoantipyrine concentration.

TABLE 28.

^{14}C-IODOANTIPYRINE UNDERESTIMATION AND RELATIONSHIP TO THE CBF (MODEL B: 60 SEC).

CBF Base line	CBF -1%	CBF -5%	CBF -10%
50	52	54	58
100	105	110	118
150	158	167	181
200	213	226	246
250	267	285	311
300	323	345	379
350	381	408	450
400	436	469	518

CBF (ml $100g^{-1}min^{-1}$) levels related to underestimation produced by liquid scintillation analyser within a value of -1%, -5% or -10% in the ^{14}C-iodoantipyrine concentration.

TABLE 29.

^{14}C-IODOANTIPYRINE UNDERESTIMATION AND RELATIONSHIP TO THE CBF (MODEL C: 30 SEC).

CBF Base line	CBF -1%	CBF -5%	CBF -10%
50	51	54	57
100	101	107	116
150	153	164	179
200	205	221	246
250	257	282	323
300	309	345	409
350	362	414	525
400	416	495	712

CBF (ml $100g^{-1}min^{-1}$) levels related to underestimation produced by liquid scintillation analyser within a value of -1%, -5% or -10% in the ^{14}C-iodoantipyrine concentration.

TABLE 30.

^{14}C-IODOANTIPYRINE UNDERESTIMATION AND RELATIONSHIP TO CBF (MODEL C: 60 SEC).

CBF Base line	CBF -1%	CBF -5%	CBF -10%
50	51	56	60
100	101	109	122
150	155	172	266
200	210	250	370
250	264	358	-
300	327	664	-
350	398	-	-
400	481	-	-

CBF (ml $100g^{-1}min^{-1}$) levels related to underestimation produced by liquid scintillation analyser within a value of -1%, -5% or -10% in the ^{14}C-iodoantipyrine concentration. (- indicates the failure of computer to convert the new blood flow value).

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